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THE THEORETICAL GENETICS OF THE SEX RATIO[1, 2]

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University of Virginia, School of Medicine, Charlottesville, Virginia

Received May 10, 1957

SEX ratios different from one half are found from time to time in animal populations and in families within populations. It is often supposed that these ratios are due to genes affecting the primary sex ratio, that is to say the sex ratio at fertilization. In certain cases the existence of such genes has been demonstrated. In several species of Drosophila there is an X-linked gene causing males which carry it to produce almost all daughters (MORGAN, BRIDGES and STURTEVANT 1925; GERSHENSON 1928). In *Drosophila affinis* NOVITSKI (1947) has found an autosomal gene which, in the presence of this X-linked gene, leads to the production of almost all sons. In both these cases it appears that only one kind of sperm cell is produced.

If you consider all the imaginable modes of action for genes affecting the sex ratio it is clear that there are two categories.

The Drosophila type just mentioned may be called a type I sex ratio gene and occurs in connection with an X-Y system of sex determination. This type of gene operates either by causing an unequal production of the two gametes in the heterogametic sex or by causing non random fertilization.

The other kind of gene is a type II sex ratio gene. It occurs where there is no chromosomal sex determination and can best be understood by considering the simplest example. Suppose there are two alleles, *A* and *a*; and that an *AA* individual is a male and an *aa* one a female. If the heterozygote is always of the same sex, say a male, only one homozygote is possible, and we have a backcross or X-Y type of sex determination. On the other hand if the heterozygote is a hermaphrodite or if it has a certain probability of developing into either a male or a female then both homozygotes as well as the heterozygote can remain in the population. The sex ratio in the population will depend upon the gene frequencies of these two alleles. Under the same general scheme a polygenic system would be possible. In any case a type II sex ratio gene is distinguished by acting as a sex determining factor in development while a type I sex ratio gene determines the frequencies with which XX and XY zygotes are formed.

An example of type II sex ratio genes is perhaps afforded by the isopods of DE LATTIN (1952). DE LATTIN claims that in his material sex is determined by autosomal genes only. In many cases of what is called environmental sex determination, type II sex ratio genes may also play a role of greater or lesser importance.

[1] Adapted from the author's Ph.D. thesis in Zoology, University of California.

[2] Part of the printing cost of the accompanying figures has been paid by the GALTON AND MENDEL MEMORIAL FUND.

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That is to say there may be a joint determination of sex by genes and environment.

Since certain of the possible kinds of sex ratio genes have been discovered and others may reasonably be anticipated to exist, I propose to examine their expected behavior. A variety of genes differing in their mode of inheritance and effect on fitness will be considered in detail. The kind of selection each gene is subject to and consequently the effect which the presence of such a gene would have on the sex ratio of a population will be determined.

Some facts are already known about the population behavior of sex ratio genes and are set forth by GERSHENSON (1928), FISHER (1930), and SHAW and MOHLER (1953).

GERSHENSON considered the sex-linked sex ratio gene of the type found in Drosophila and which causes males carrying it to produce only daughters. He explains that such a gene should tend to increase in frequency. Experiments by WALLACE (1948) show that the effects on fitness produced by the *sex ratio* factor in *Drosophila pseudoobscura* are also important in determining the population behavior of this gene.

FISHER dealt with the relationship between parental care and the sex ratio. His discussion is difficult to paraphrase and should be consulted in the original. He is mainly concerned in showing that with parental care and a difference in viability between the sexes before they leave the care of the parents, the primary sex ratio will be adjusted to yield an excess of the less viable sex. This principle merits special attention since it may hold for man. Nevertheless, it cannot be considered here. In the examples to be given parental care is assumed to be absent or to play a negligible role.

It would seem to follow from FISHER's treatment that where there is no parental care or where offspring of the two sexes are equally viable the sex ratio will be adjusted to one half. This is the conclusion that MOHLER and I came to using a different approach. The conclusion is broadly applicable to the sex ratio insofar as it is determined by autosomal genes.

An approach different from any of the previous ones is used here. If it is assumed that the sex ratio is under the control of a given genetic mechanism, the successive frequencies of the genotypes present can be calculated, and so can the sex ratio of the population in each generation. Iterative calculations of this type are known to every geneticist. What follows is only a method for facilitating this kind of generation-by-generation calculation.

The figures for these calculations are written as matrices. The term "matrix" merely means a rectangular array of numbers and is legitimately used here even though no matrix algebra is employed.

Definitions:

a) The genotypes among the males are designated 1, 2, 3 . . . i . . . n, and among the females 1, 2, 3 . . . j . . . k.

b) The probability of genotype i among the males is z_i and of j among the females z_j.
c) The fitness of genotype i is w_i, a value such that rw_i gives the expected number of zygotes which will be produced by an individual (zygote) of genotype i. The factor r is a constant. The fitness of a female, w_j, is defined in the same fashion.
d) The initial number of male zygotes in the population is M and that of female zygotes, F.

Derivation:

Given the values of z_i and z_j in the parental generation we can find the corresponding values in the offspring generation.
1) The parental zygotes of genotype i, taken collectively and over their whole lives, are expected to produce rw_iMz_i offspring zygotes.
2) The probability that a zygote selected at random from the offspring generation is from an i father and also from a j mother, under the assumption of random mating, is

$$\frac{rw_iz_iM}{\Sigma\, rw_iz_iM} \cdot \frac{rw_jz_jF}{\Sigma\, rw_jz_jF}$$

$$= \frac{w_{ij}z_{ij}}{\Sigma\Sigma\, w_{ij}z_{ij}}$$

3) The probability that a zygote is i given that its parents are ij may be designated $s^{(i)}{}_{ij}$ and the probability that it is j, $s^{(j)}{}_{ij}$. (No summation applies to the (i) or (j) superscripts unless explicitly indicated, as for example, by $\sum_{(i)=1}^{n}$.)

4) The probability in the offspring generation of an individual which is both i and from ij parents is

$$\frac{(zw)_{ij}}{\Sigma\Sigma\,(zw)_{ij}} \cdot s^{(i)}{}_{ij} = \frac{(zws^{(i)})_{ij}}{\Sigma\Sigma\,(zw)_{ij}}$$

5) The probability of genotype i is the sum of the above or

$$[\Sigma\Sigma\,(zw)_{ij}]^{-1}\,\Sigma\Sigma\,(zws^{(i)})_{ij}\ .$$

6) The probability of a male zygote given that the parents are ij may be defined as m_{ij}, and the probability of a female zygote as f_{ij}. (Note that the matrices $[m_{ij}]$ and $[f_{ij}]$ can be gotten as the sums of certain other matrices since they are the same as $\sum_{(i)=1}^{n} s^{(i)}{}_{ij}$ and $\sum_{(j)=1}^{k} s^{(j)}{}_{ij}$).
7) The probability of a male zygote in the offspring generation is

$$\frac{(zw)_{ij}}{\Sigma\Sigma\,(zw)_{ij}} \cdot m_{ij} = [\Sigma\Sigma\,(zw)_{ij}]^{-1}[\Sigma\Sigma\,(zwm)_{ij}]$$

8) The probability of genotype i among the offspring males is

$$z'_i = \frac{P\{i\}}{P\{\delta\}}$$

$$= \frac{[\Sigma\Sigma\,(zw)_{ij}]^{-1}[\Sigma\Sigma\,(zws^{(i)})_{ij}]}{[\Sigma\Sigma\,(zw)_{ij}]^{-1}[\Sigma\Sigma\,(zwm)_{ij}]}$$

$$z'_i = \frac{\Sigma\Sigma\,(zws^{(i)})_{ij}}{\Sigma\Sigma\,(zwf)_{ij}}$$

and likewise for the females

$$z'_j = \frac{\Sigma\Sigma\,(zws^{(j)})_{ij}}{\Sigma\Sigma\,(zwf)_{ij}} \,.$$

Remember that z'_i and z'_j represent the zygotic frequencies in the succeeding generation of the various genotypes and that i and j indicate the arbitary number given to each genotype for identification. The iterative use of these equations will give the successive genotype frequencies for any desired number of generations.

A slight improvement in computation can be effected by multiplying the two equations together to give

$$z'_{ij} = \frac{\Sigma\Sigma\,(zws^{(i)})_{ij}\ \Sigma\Sigma\,(zws^{(j)})_{ij}}{\Sigma\Sigma\,(zwm)_{ij}\ \Sigma\Sigma\,(zwf)_{ij}} \,.$$

Directions for computing an actual example will now be given in order to show more concretely what the various symbols mean and how a numerical result is obtained. Suppose that the sex ratio is influenced by a certain locus and that the recessive gene a causes males to produce all sons rather than half sons and half daughters. The matrix for the probability of an aa male is

$[s^{(i)}{}_{ij}] =$

♂ ♂ \ ♀ ♀	AA	Aa	aa
AA	0	0	0
Aa	0	⅛	¼
aa	0	½	1

This matrix gives the probability, for each different possible parental combination, of an offspring that is aa and a male. The letter s is an appropriate symbol

for this type of matrix because its elements are probabilities due to *segregation*. The superscript is given as $(i) = 1$ in accordance with the assignment of numbers to the genotypes and could also be written $(i) = aa$. All the matrices of this kind are written out below but without the complete labelling for parental combinations.

$$[s_{ij}{}^{(i)=1}] = \begin{matrix} \\ \\ \\ \end{matrix} \begin{matrix} \text{♂ ♂} \\ \end{matrix} \begin{bmatrix} \frac{1}{2} & \frac{1}{4} & 0 \\ \frac{1}{4} & \frac{1}{8} & 0 \\ 0 & 0 & 0 \end{bmatrix} \qquad [s_{ij}{}^{(j)=1}] = \begin{matrix} \\ \\ \\ \end{matrix} \begin{matrix} \text{♀ ♀} \\ \end{matrix} \begin{bmatrix} \frac{1}{2} & \frac{1}{4} & 0 \\ \frac{1}{4} & \frac{1}{8} & 0 \\ 0 & 0 & 0 \end{bmatrix}$$

$$[s_{ij}{}^{(i)=2}] = \begin{bmatrix} 0 & \frac{1}{4} & \frac{1}{2} \\ \frac{1}{4} & \frac{1}{4} & \frac{1}{4} \\ 1 & \frac{1}{2} & 0 \end{bmatrix} \qquad [s_{ij}{}^{(j)=2}] = \begin{bmatrix} 0 & \frac{1}{4} & \frac{1}{2} \\ \frac{1}{4} & \frac{1}{4} & \frac{1}{4} \\ 0 & 0 & 0 \end{bmatrix}$$

$$[s_{ij}{}^{(i)=3}] = \begin{bmatrix} 0 & 0 & 0 \\ 0 & \frac{1}{8} & \frac{1}{4} \\ 0 & \frac{1}{2} & 1 \end{bmatrix} \qquad [s_{ij}{}^{(j)=3}] = \begin{bmatrix} 0 & 0 & 0 \\ 0 & \frac{1}{8} & \frac{1}{4} \\ 0 & 0 & 0 \end{bmatrix}$$

$$[m_{ij}] = \begin{bmatrix} \frac{1}{2} & \frac{1}{2} & \frac{1}{2} \\ \frac{1}{2} & \frac{1}{2} & \frac{1}{2} \\ 1 & 1 & 1 \end{bmatrix} \qquad [f_{ij}] = \begin{bmatrix} \frac{1}{2} & \frac{1}{2} & \frac{1}{2} \\ \frac{1}{2} & \frac{1}{2} & \frac{1}{2} \\ 0 & 0 & 0 \end{bmatrix}$$

If we suppose that *aa* females have a fitness of one half while the other genotypes are unaltered in fitness, the matrix for fitness is

$$[w_{ij}] = \begin{bmatrix} 1 & 1 & \frac{1}{2} \\ 1 & 1 & \frac{1}{2} \\ 1 & 1 & \frac{1}{2} \end{bmatrix}$$

Finally suppose that the three genotypes occur in frequencies 0.3, 0.3, 0.4 in the males and 0.3, 0.4, 0.3 in the females. Then the z matrix together with its marginal totals, which are given for clarity, is

$$[z_{ij}] = \begin{bmatrix} .09 & .12 & .09 \\ .09 & .12 & .09 \\ .12 & .16 & .12 \end{bmatrix} \begin{matrix} .30 \\ .30 \\ .40 \end{matrix}$$

$$\begin{matrix} .30 & .40 & .30 \end{matrix}$$

For ease in calculation it is desirable to combine the w matrix with each of the segregation matrices by multiplying corresponding elements. Whether this is done or not the formula

$$z'_{ij} = \frac{\Sigma\Sigma\,(zws^{(i)})_{ij}\;\Sigma\Sigma\,(zws^{(j)})_{ij}}{\Sigma\Sigma\,(zwm)_{ij}\;\Sigma\Sigma\,(zwf)_{ij}}$$

can now be applied. The first step is to calculate the denominator for which there is only one value. Next calculate the numerator for all the values of i and j and arrange the figures in their proper order in an i by j matrix. Multiply each element of the matrix by the reciprocal of the denominator (because with a desk calculator this is easier than dividing). The result is the matrix z'_{ij} for the succeeding generation. The frequencies of the various genotypes are gotten from this simply by taking the marginal totals. This can be done at each generation, at intervals of several generations, or only for the final generation.

Examples:

Various examples will now be considered. Each of these is given as a figure captioned so as to be self-explanatory, and each illustrates the behavior of a different kind of sex ratio gene. A sex ratio gene is always designated by the letter a either capitalized or lower case depending on dominance. A normal gene is designated by $+$.

The initial frequencies of the genotypes for all examples are selected so as to be quite different from the apparent limiting values. The gene's behavior over a wide range of frequencies can thus be observed. Many of the graphs show violent fluctuations for the first few generations. This is due to the arbitrary initial frequencies.

Figures 1, 2, 3, and 4 are concerned with autosomal genes. In these very simple cases all values of w are 1, and the sex ratio gene is in competition only with a normal allele. The sex ratio gene is progressively reduced in frequency and the more rapidly so when it is dominant.

Figure 5 is for two alleles with opposite effects on the sex ratio. An equilibrium is reached with the sex ratio of the population at one-half.

Figures 6 and 7 show an autosomal dominant which has effects other than on the sex ratio. The way in which fitness is altered is the same in both of these except that in Figure 7 the reduction of fitness is in the female and in Figure 6 in the male. The results are quite different. In Figure 7 it is not clear from the graph whether or not an equilibrium will eventually be established with both alleles remaining in the population. In Figure 6 this has actually occurred after 11 generations.

In Figure 7 equilibrium is not established at an intermediate value. The frequencies all go to zero or one. This can be shown as follows. The $A/+$ females can come only from the cross $+/+\ \delta$ by $A/+\ \varphi$, but half the female zygotes from that cross will be $+/+$. The frequency of $A/+$ females will thus decline leaving, eventually, only $+/+$ females. This leads immediately to the consequence that

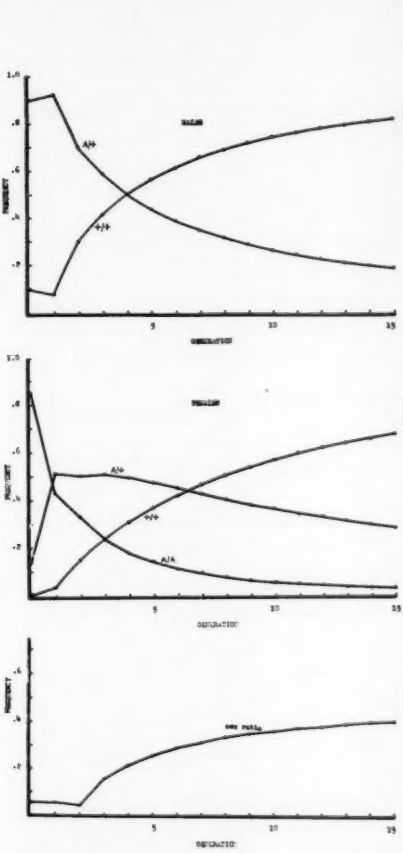

FIGURE 1.—An autosomal dominant causing males to produce only daughters.

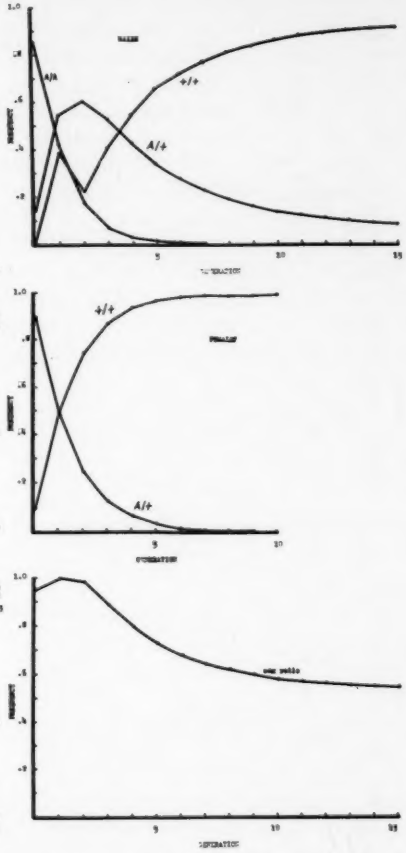

FIGURE 2.—An autosomal dominant causing males to produce only sons.

no A/A males can appear. There now remain in the population only one kind of female but two kinds of male: $A/+$ and $+/+$. The $A/+$ males will also be gradually eliminated due to their property of producing half $+/+$ sons.

The importance of Figures 6 and 7 is this. The effect on the sex ratio tends to reduce the frequency of the gene while the effect on fitness is such as to increase it. It might be expected that such circumstances would lead to an equilibrium with a gene frequency somewhere between zero and one. WALLACE supposed the existence of such a stable equilibrium for the sex-linked sex ratio gene in *Drosophila pseudoobscura*, and his experiments support this supposition. Nevertheless, as a

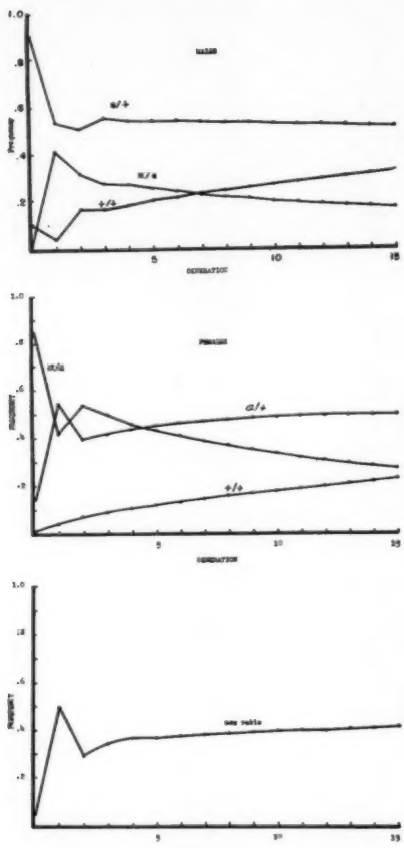

FIGURE 3.—An autosomal recessive causing males to produce only daughters.

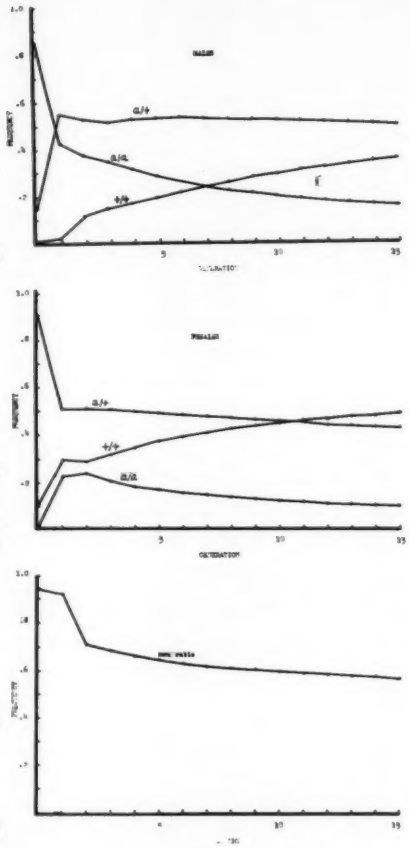

FIGURE 4.—An autosomal recessive causing males to produce only sons.

general principle, the presence of two opposing forces is not necessarily sufficient to establish a stable equilibrium at an intermediate gene frequency. Figures 6 and 7 show that such an equilibrium for sex ratio genes is possible but not invariable.

Figure 8 shows a type II sex ratio gene. Its effect is such that a zygote receiving this gene is certain to develop into a male, while one not receiving it has a probability of one half of so doing. The outcome is that the strong male determining gene is eliminated and the sex ratio of the population approaches one half.

Figure 9 is an example of two alleles, each producing an abnormal sex ratio.

They have opposite effects and both alleles remain in the population which is stabilized with the attainment of an over-all sex ratio of one half.

Many animals which are said to have environmental sex determination may really have a sex determination which is partly genetic. Besides the effect of the environment there may be genes affecting the lability of change toward one sex or the other. This possibility is supported by the contention of COE (1948) respecting the protandrous gastropod, *Crepidula plana*. In this species young individuals living near females develop into males and retain that condition for some time, subsequently becoming females. Those which are not near females undergo only a brief male phase or none. There are, however, exceptions. Some individuals associated with females retain the male phase throughout their lifetimes. Others, although isolated, develop a full male phase. COE suggests that these individuals are genetically different from the other members of the species. Although the case of Crepidula is complicated by alternating sexuality, the facts are

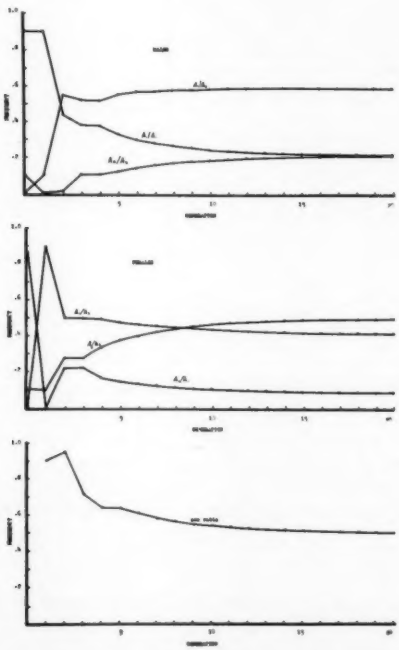

FIGURE 5.—Two alleles with opposite effects on the sex ratio. A_1/A_1 fathers produce only sons; A_1/A_2 fathers produce half sons, half daughters; A_2/A_2 fathers produce only daughters.

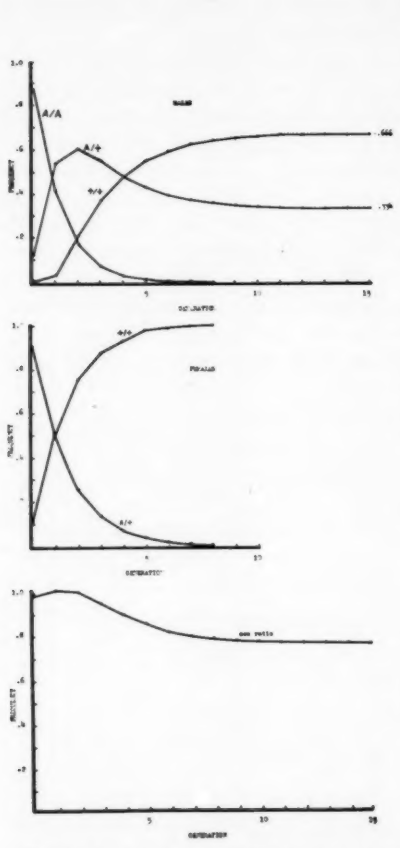

FIGURE 6.—An autosomal dominant causing males to produce only sons. Fitness values for *A/A*, *A/+*, and *+/+* males are respectively 1, 1, ½. In the females all three genotypes are equally fit.

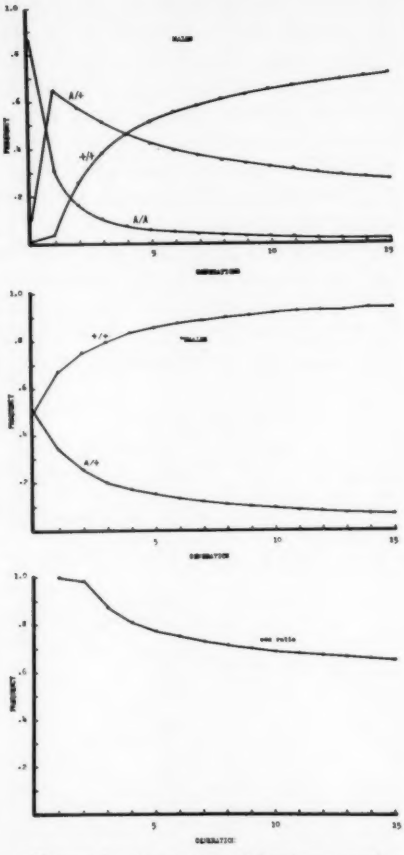

FIGURE 7.—An autosomal dominant causing males to produce only sons. Fitness values for *A/A*, *A/+*, and *+/+* females are respectively 1, 1, ½. In the males all genotypes are equally fit.

conducive to the belief that genes modifying environmental sex determination do exist.

If the interpretation of DE LATTIN is correct, his isopods have a kind of sex determination in which there are no sex chromosomes, but also no effect of environment. Instead of being determined partly by environmental and partly by genetic factors, sex is fully determined by a set of genes. These genes, of course, are all autosomal. Genes of this kind are very similar to those in Figures 8 and 9.

Both genes modifying environmental sex determination and those of the sort suggested by DE LATTIN are type II sex ratio genes and therefore similar to those of Figures 8 and 9. The examples suggest that a population with type II sex ratio genes will have an equilibrium sex ratio of one half. (If temporal fluctuations in environment are important, the sex ratio might, of course, fluctuate around this value.) Whether this holds as a general principle can be investigated using an approach similar to that of FISHER.

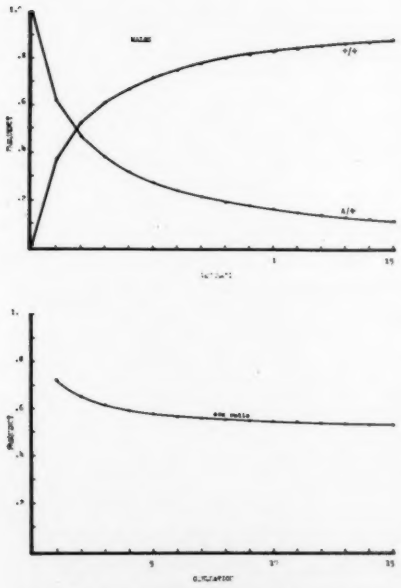

FIGURE 8.—An autosomal dominant type II sex ratio gene. The probability that a zygote becomes a male is 1 if it has an A gene, ½ if it has only $+$.

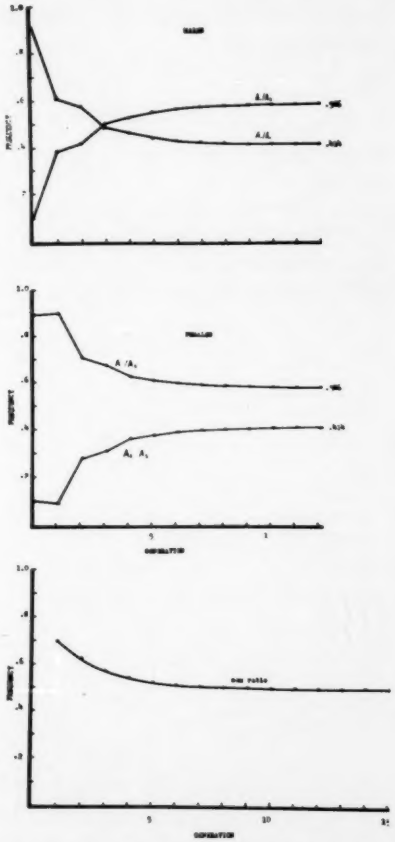

FIGURE 9.—An autosomal type II sex ratio gene. The probability that a zygote becomes a male is 1, ½, or 0 depending on whether its genotype is respectively A_1/A_1, A_1/A_2, or A_2/A_2.

Suppose that the sex ratio is under genic control and that none of the genes are sex-linked—in fact the organism considered has no sex chromosomes. If there are N zygotes in a generation and NM of them are males, then one male zygote is expected to contribute $\frac{1}{2} \cdot 1/NM$ or $1/2NM$ of the genes going to the next generation. Likewise a female zygote is expected to contribute $1/2NF$. These "contributions" may be said to be the genetic value of a male and of a female zygote respectively. Equilibrium must occur when the value of a male zygote is equal to that of a female. This is so because if the values were not equal, parents with genotypes leading to the production of mainly the more valuable sex would be favored over the others. Selection would thus proceed. Equal zygotic values of the sexes means that

$$\frac{1}{2NM} = \frac{1}{2NF} \quad .$$

From this, M must equal F showing that equilibrium is attained at a sex ratio of one half.

Provided this requirement is met, a population may remain heterogeneous for type II sex ratio genes. This is just what has been claimed by DE LATTIN whose populations of isopods were of two color patterns. Strains of these two raised in the laboratory had sex ratios of .68 and .32. Equilibrium would be expected where

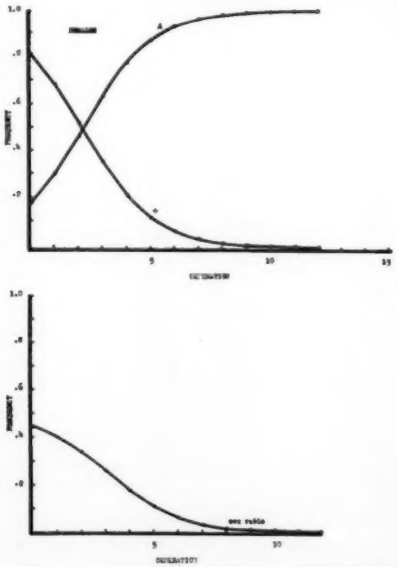

FIGURE 10.—A Y-linked gene causing females to produce only daughters. The female is the heterogametic sex.

the sex ratio of the whole population is one half. This would occur with the two types in equal numbers. Although there were differences from one population to another, DE LATTIN did indeed find the two types in very nearly equal frequencies in natural populations.

A completely Y-linked gene is considered in Figure 10. The female is heterogametic, and the sex ratio gene causes females carrying it to produce all daughters. Here the sex ratio gene increases to fixation. It is not difficult to see what happens. Females carrying the gene give a Y chromosome to every offspring while the normals give a Y to only half. The favored allele at a Y-linked locus is the one that leads to the production of the greatest proportion of the heteroga-

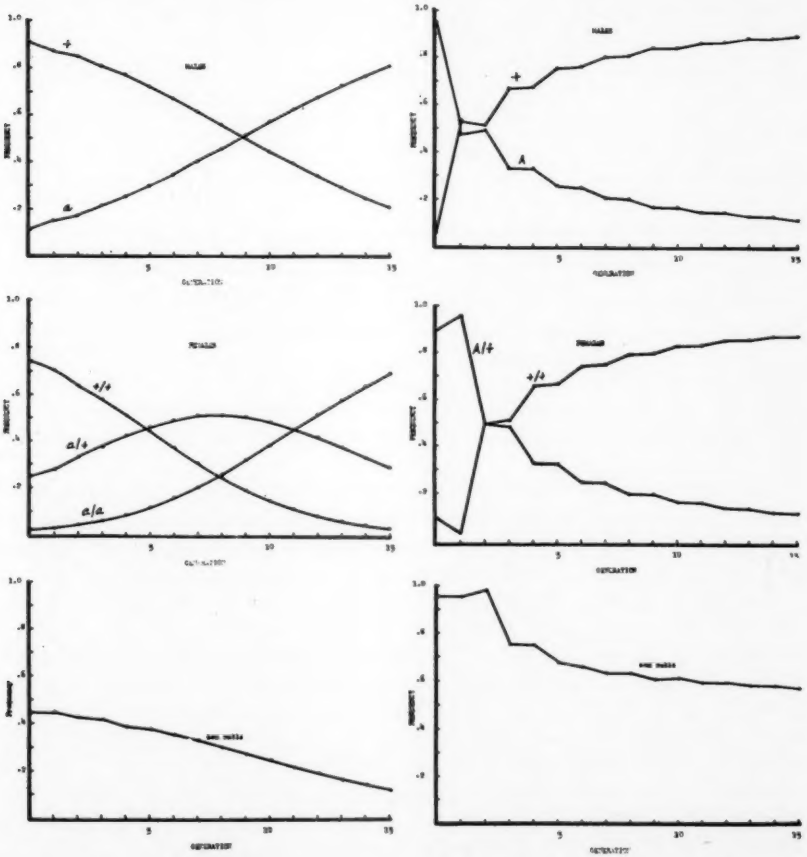

FIGURE 11.—An X-linked recessive causing males to produce only daughters.

FIGURE 12.—An X-linked dominant causing females to produce only sons.

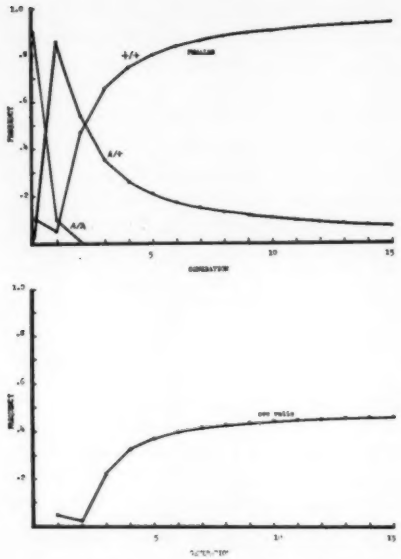

FIGURE 13.—An X-linked dominant causing females to produce only daughters. The frequency of *A* goes to zero in the first generation in the males.

metic sex. Clearly a population cannot remain heterogeneous for Y-linked sex ratio genes unless it is maintained so by fitness differences which counteract the type of selection just described.

HOWARD (1942) describes three kinds of females in the land isopod *Armadillium vulgare*: females which produce nearly all daughters, females which produce nearly all sons, and females which produce sons and daughters in equal numbers. He suggests that this condition is probably due to cytoplasmic factors, but perhaps to genes on the Y chromosome. (The female is presumably heterogametic.) The hypothesis of genes on the Y chromosome can certainly be eliminated. A gene for the production of nearly all daughters would rapidly approach fixation. The gene for mostly sons would very rapidly disappear. If it led to the production of exclusively sons, it would disappear entirely in one generation. The resulting picture is similar to that of Figure 10. The fitness differences necessary to maintain an equilibrium would be extremely high.

Figures 11, 12, and 13 are of sex-linked genes.

Figure 11 is of the kind of gene known in various species of Drosophila. As indicated by GERSHENSON, it tends to increase in the population.

Figures 12 and 13 are of sex-linked genes acting in the female. In both of these examples the gene tends to be eliminated.

A peculiarity of sex ratio genes can be seen in the examples which have been calculated. The zygotic gene frequency as well as the frequencies of the genotypes may be different in the two sexes. X-linked genes under selection can have different zygotic frequencies in the two sexes even when there is no effect on the sex ratio; autosomal genes, except those subject to non random disjunction, cannot.

SUMMARY

A method is given for calculating the genotype frequencies in successive generations in a population with genetic variability of the sex ratio. By computing specific examples the population behavior of genes affecting the sex ratio is investigated.

ACKNOWLEDGEMENT

I wish to thank PROF. CURT STERN, under whose direction the work was done, for having encouraged me to pursue this problem.

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THE EFFECT OF TRYPTOPHAN ON THE PENETRANCE OF TUMOROUS HEAD IN CROSSES AMONG SELECTED STOCKS OF DROSOPHILA MELANOGASTER*

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THE tumorous head phenotype and genetic mechanism have been described by several investigators (GARDNER 1948; GARDNER and WOOLF 1949; NEWBY 1949; GARDNER and RATTY 1952). Two major genes associated with the trait were located from linkage data in the first and third chromosomes (STOTT and GARDNER 1952). A maternal effect (GARDNER and GARDNER 1953) was detected from reciprocal outcrosses. Modifiers (GARDNER and STOTT 1951; GARDNER, STOTT and DEARDEN 1952) were found to be widespread in laboratory stocks and wild populations. Alleles of the sex-linked gene were identified in three laboratory stocks. Increased temperature (GARDNER and WOOLF 1950) from 18° C to 30° C was associated with a progressive increase in penetrance. A temperature effective period was identified during the first 24 hours of development.

PLAINE and GLASS (1955) showed that L-tryptophan added to a complete food medium increased the expression of larval melanotic tumors and also the erupt eye phenotype which is similar to one expression of tumorous head. Other investigators (HINTON *et al.* 1951; MITTLER 1952) have also reported that tryptophan in high concentrations produces phenotypic abnormalities including multiple melanotic tumors and eye malformations. Hydrogen peroxide (PLAINE 1955a) increased oxygen tension and X-rays (PLAINE 1955b; GLASS and PLAINE 1952; PLAINE and GLASS 1952) also increased tumor incidence in the suppressor-erupt stock. The action of X-rays on tryptophan was more pronounced when combined with increased oxygen tension. A considerable amount of information on the metabolism of tryptophan has been accumulated (KIKKAWA 1950, 1953; MEHLER 1955; PLAINE 1955; WHITE *et al.* 1954). The present study was undertaken to determine the effect of L-tryptophan and related compounds on the tumorous head phenotype and the maternal effect.

MATERIALS AND METHODS

The inbred tumorous head (*tu-h*) stock maintained in this laboratory for several years was used in the experiments. Other laboratory stocks involved in the crosses were Canton and Stephenville. Canton had been shown to behave like most other stocks when crossed with *tu-h*. Stephenville had been shown to carry an allele (*tu-1*^{s}) of one of the *tu-h* genes (*tu-1*). A derived Stephenville stock

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which did not carry *tu-1*^{s} was also used in the experiments. The Stephenville stock carrying *tu-1*^{s} was identified as Stephenville^{1} and the stock without *tu-1*^{s} was designated Stephenville^{2}.

Before testing the effect of L-tryptophan and other chemicals it was necessary to determine the present penetrance level of tumorous head under normal laboratory conditions. It was also necessary to explore the relation between the age of the culture and the penetrance level. First a series of six cultures was prepared from the inbred *tu-h* stock and maintained in half pint bottles at room temperature (23°–28° C) on the standard corn meal-molasses-agar medium. The progeny were removed at 24-hour intervals and examined under a magnification of 45×. Flies showing any irregularity of the eyes or other parts of the head were classed as abnormal. Some bottles were held from 27 to 43 days to observe the effect of the age of the culture on penetrance.

For the second series, Petri dishes were prepared with the standard laboratory medium. Large numbers of males and females expressing the tumorous head trait were selected from the inbred stock bottles and placed in ring laying cages 7.5 cm in diameter and 1 cm high, similar to those used by KING (1955). The ring was covered with dacron netting which was fine enough to retain the flies but allowed access to the food for oviposition. Cultures were kept moist in a constant temperature incubator at 25 ± 1° C. At 24-hour intervals the parent flies were transferred to fresh dishes. The adult flies which emerged from the cultures were etherized and examined in the usual way. By use of the laying cages it was possible to maintain the same group of parents for the 41-day period of the experiment and thus to insure a uniform genetic background for all experimental cultures. Environmental conditions were controlled as well as possible to maintain uniform conditions and thus eliminate many environmental variables.

For the treatment with tryptophan and other chemicals, brewer's yeast was used in the medium and live yeast was not added. Brewer's yeast was shown by PLAINE (1952) to supply a complete medium for Drosophila. The control medium was prepared with 5 percent brewer's yeast and 1.5 percent agar. Compounds to be tested were added to this medium. A 0.5 percent concentration of tryptophan was used, higher concentrations, in solution, having been shown in preliminary experiments and by PLAINE and GLASS (1955) to be toxic. Indole was added at 0.01 percent, DL-serine at 0.5 percent and L-cysteine at the 0.1 percent level.

Crosses employed to test the effects of the chemicals on the penetrance of tumorous head and the maternal effect were as follows (female listed first): Canton × Canton, Canton × *tu-h*, *tu-h* × Canton, Stephenville^{1} × Stephenville^{1}, Stephenville^{1} × *tu-h*, *tu-h* × Stephenville^{1}, Stephenville^{2} × Stephenville^{2}, Stephenville^{2} × *tu-h*, *tu-h* × Stephenville^{2}, and *tu-h* × *tu-h*. Four to six replications were included in each experiment.

EXPERIMENTAL RESULTS

The results of experiments designed to determine the present penetrance level of the tumorous head stock are summarized in Table 1. From the total of 2382

flies classified from the same six cultures at daily intervals for 13 days an average of 93.4 percent showed the tumorous head phenotype. Among the 1097 females 93.5 percent were abnormal compared with 93.3 percent for the 1285 males. Considerable variation was observed in the penetrance represented by the daily counts of individual cultures. Penetrance values above the mean and those considerably below were usually associated with small numbers of flies. When averages were considered over the 13-day period, the penetrance levels were consistent.

TABLE 1

Penetrance in inbred tu-h *cultures at room temperature. Averages of six separate cultures from which all flies were removed daily*

24-hour interval	Total flies classified	Avg. % abn. (6 cultures)
1	42	90
2	271	96
3	308	94
4	373	96
5	333	93
6	237	93
7	146	94
8	168	95
9	143	83
10	126	92
11	95	93
12	71	93
13	69	93
	2382	93.4

When the flies were not removed from the cultures each day but were allowed to remain in the bottles for long periods of time, the penetrance decreased. Bottles 27 days old had an average of 74 percent abnormal flies. Those 38 days old showed an average of 67 percent and those 43 days old 61 percent. None of the individual cultures 27 days old or older had a penetrance exceeding 76 percent.

The next experiment, designed to investigate the cause of the decrease in penetrance associated with culture age, was carried out with laying cages. Parent flies from the inbred *tu-h* stock were transferred daily for 41 days to new food and allowed to deposit eggs. The number of eggs laid in the different 24-hour intervals varied considerably, but sufficient numbers were obtained during each period to provide meaningful penetrance values. From the total of 2664 adult flies classified from the 41 cultures, an average of 96.1 percent was abnormal. Among the 1278 females 96.6 percent and among the 1386 males 95.7 percent was abnormal. The cultures in the lower range were distributed through the 41-day period. Average penetrance levels were as high at the end of the experiment as at the beginning, indicating that the age of the parents had no effect on the penetrance level in the progeny.

For testing the effects of L-tryptophan, indole, DL-serine, and L-cysteine, reciprocal crosses involving *tu-h* and wild stocks, Canton and Stephenville, were carried along with the four inbred line crosses; i.e., Canton, Stephenville[1], Stephenville[2], and *tu-h*. Reciprocal crosses were used to detect any influence which the chemicals might have on the maternal effect which would be observable in the F_1. The results of the control crosses maintained on the brewer's yeast-agar medium are summarized in Table 2. The maternal effect was indicated in all crosses in which *tu-h* or Stephenville[1] females were involved. From the cross between *tu-h* females and Canton males for example, 39 percent of the progeny was abnormal. No abnormal flies were observed in the results of the reciprocal cross between Canton females and *tu-h* males.

TABLE 2

Penetrance of tumorous head among F_1 progeny from different crosses maintained on control medium

Cross†	Females		Males		Total	
	n*	%‡	n	%	n	%
Can × Can	311	0	278	0	589	0
Can × *tu-h*	453	0	507	0	960	0
tu-h × Can	487	42	417	35	904	39
Steph[1] × Steph[1]	442	0	490	0	932	0
Steph[1] × *tu-h*	395	46	452	47	847	47
tu-h × Steph[1]	206	62	156	41	362	53
Steph[2] × Steph[2]	477	0	435	0	912	0
Steph[2] × *tu-h*	417	0	391	0	808	0
tu-h × Steph[2]	257	51	210	33	467	43
tu-h × *tu-h*	216	96	320	94	536	95

† The female parent is listed first in each cross.
*n = total number of flies.
‡% = percentage of abnormal flies to the nearest whole number.

When Stephenville[1] females were mated with the *tu-h* males 47 percent of the progeny was abnormal. From the reciprocal cross between the *tu-h* females and the Stephenville[1] males 53 percent was abnormal. When *tu-h* females were crossed with Stephenville[2] males 43 percent of the progeny was abnormal. No abnormal flies resulted from the reciprocal cross between Stephenville[2] females and *tu-h* males. The inbred *tu-h* females crossed with males from the same stock produced 95 percent abnormal progeny. The development of the flies on the brewer's yeast-agar medium was normal, with eclosion occurring at the usual time. Ample food was available in each culture throughout the developmental period. Penetrance levels in the *tu-h* × *tu-h* control were comparable with those of the preliminary experiments cited above.

When the same crosses were made and the cultures were maintained under the same environmental conditions except that 0.5 percent L-tryptophan was added to the medium, the penetrance was significantly decreased. The results are summarized in Table 3. Averages of 22, 21, 28, 30, and 71 percent penetrance were

obtained from the crosses: *tu-h* × Canton, Stephenville[1] × *tu-h*, *tu-h* × Stephenville[1], *tu-h* × Stephenville[2], and *tu-h* × *tu-h*, respectively. From the crosses involving *tu-h* or Stephenville[1] females the penetrance levels were about 20 percent below those of the controls with no additive in the medium. By using 2 × 2 contingency tables, the results were tested with the chi-square method for goodness of fit. The differences were significant at the one percent level. It is, therefore, improbable that the differences between the control and treatment percentages were due to chance alone. The addition of 0.5 percent L-tryptophan to a complete medium decreased the penetrance of the tumorous head phenotype.

About half of the tryptophan treated progeny from the Stephenville × Stephenville crosses had melanotic bodies in their abdomens. These dark bodies were also observed in the abdomens of about ten percent of the tryptophan treated progeny of outcrosses involving Stephenville. The bodies were subcutaneous and scattered throughout the abdominal region. The size and number varied considerably. The

TABLE 3

Penetrance of tumorous head among F_1 progeny from different crosses maintained on control medium with 0.5 percent L-tryptophan added

Cross†	Females		Males		Total	
	n*	%‡	n	%	n	%
Can × Can	135	0	168	0	303	0
Can × *tu-h*	274	0	245	0	519	0
tu-h × Can	279	29	240	13	519	22
Steph[1] × Steph[1]	39	0	43	0	82	0
Steph[1] × *tu-h*	220	19	157	24	377	21
tu-h × Steph[1]	79	33	66	23	145	28
Steph[2] × Steph[2]	233	0	214	0	447	0
Steph[2] × *tu-h*	376	0	333	0	709	0
tu-h × Steph[2]	169	37	181	24	350	30
tu-h × *tu-h*	250	73	346	70	596	71

† The female parent is listed first in each cross.
*n = total number of flies.
‡% = percentage of abnormal flies to the nearest whole number.

dark bodies could be identified in late third instar larvae and early pupal stages and also in the adult flies. Some mature flies with dark bodies were transferred to the usual laboratory corn meal-molasses-agar medium. The melanotic bodies were maintained in these flies but did not appear in any of their progeny. As long as the Stephenville flies were kept on the medium with the tryptophan additive, the dark bodies persisted generation after generation. They were not observed in cultures other than Stephenville which were treated with tryptophan. The melanotic bodies were thus dependent on something in the Stephenville strain and were expressed only in the presence of excess tryptophan.

When indole was added to the complete medium in a concentration of 0.01 percent the penetrance level was not altered. The statistical analysis based on the chi-square method indicated that the deviations from the controls were not signifi-

cant. Likewise, the addition of 0.5 percent DL-serine to the medium produced no significant effect on the penetrance of tumorous head. Treatment with L-cysteine at 0.1 percent concentration gave a varying effect. The crosses *tu-h* × Canton and *tu-h* × Stephenville showed no statistically significant differences as compared with the controls. The effect of L-cysteine on the *tu-h* × *tu-h* crosses was more pronounced. The average percentage of abnormal flies was 79 percent as compared with 95 percent for the controls. The chi-square test indicated that the difference was significant at the one percent level.

DISCUSSION

When the tumorous head stock was first investigated, a penetrance of 64 percent was reported. Following close inbreeding and selection over a two-year period, the penetrance was increased to 79 percent. The present experiments place the penetrance level at 93 percent. The best explanation for the increase following inbreeding and rigid selection is based on the accumulation of modifiers favorable to the expression. Modifiers of tumorous head have been shown to be widespread in wild populations and laboratory stocks. Continued selection, required to maintain this stock, would tend to concentrate the modifiers favorable to the expression. Close inbreeding would tend to raise the frequency of favorable modifiers and they would become homozygous. Segregation of heterozygous modifiers could in part, explain the variability which has been observed in the expression of the tumorous head phenotype in stock cultures and outcrosses.

The results obtained from the experiments on penetrance in the inbred stock emphasize the importance of the environment in determining the penetrance level. Given abundant food, uncrowded cultures, favorable temperature and humidity the penetrance level is high and there is a relatively small variation from culture to culture. In older cultures where there is crowding and competition for the available food, the penetrance level is lower and highly variable. In view of the low viability of *tu-h* as compared with wild flies, (GARDNER and RATTY 1952) it is probable that the decrease in the percentage of abnormal flies found in older cultures is due primarily to the failure of the tumorous head flies to survive until the time of classification rather than an increase in the number of normal flies hatched.

The appearance of melanotic growths in the Stephenville flies is interesting in view of the observations of HINTON *et al.* (1951), MITTLER (1952) and PLAINE and GLASS (1955) that tryptophan in excess increases the incidence of certain types of melanotic growths in particular stocks studied by these investigators.

In the *tu-h* inbred stock and in the results and outcrosses involving *tu-h* and Stephenville[1] females the incidence of the tumorous head abnormality was significantly lowered when tryptophan was added to the medium. It would be presumptuous, without further investigation, to attribute this lowered penetrance to a direct effect of tryptophan on the genetic-physiological mechanism of the *tu-h* phenotype. In view of the lower viability of the *tu-h* strain as compared with wild stocks, it is quite possible that the decreased penetrance could result from a dif-

ferential toxic effect of tryptophan on the flies expressing the tumorous head phenotype. This possibility is being investigated.

While tryptophan was not found to increase the penetrance of the tumorous head phenotype, there is a possibility that other substances might. Compounds of the relatively simple nature of L-tryptophan could hardly be expected to influence all tumor-like growths in *Drosophila melanogaster*. The recent report of BURTON, HARNLY and KOPAC (1956) of an extract from a melanotic tumor-bearing stock which is effective in inducing growths in non-tumorous strains indicates that substances of a more complex nature may be involved in the production of some types of abnormal growths.

SUMMARY

1. The present penetrance level of the inbred tumorous head stock was found to average 93 percent when the cultures were maintained on the standard medium at room temperature.

2. The age of the parental flies did not affect the penetrance level, but in cultures allowed to develop for an extended period without removal of flies the penetrance level decreased. The lowered penetrance in old cultures probably resulted from the inability of the tumorous head flies to compete favorably against those of normal phenotype in the same cultures.

3. When flies were reared on a brewer's yeast-agar medium to which 0.5 percent tryptophan was added, the penetrance of tumorous head was significantly reduced.

4. When 0.5 percent tryptophan was added to the medium of flies from the Stephenville stock, about half of the progeny had melanotic bodies in their abdomens. In the same medium about 10 percent of the progeny from outcrosses involving Stephenville had similar melanotic bodies.

5. Indole at 0.01 percent concentration and DL-serine at 0.5 percent did not significantly affect the penetrance of the tumorous head phenotype.

6. L-cysteine at a concentration of 0.1 percent did not significantly affect the results of the outcrosses tested but the penetrance of the tumorous head phenotype was significantly reduced in the inbred *tu-h* flies when 0.1 percent L-cysteine was added to the medium.

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A GENETIC ANALYSIS OF SOME REARRANGEMENTS INDUCED IN OOCYTES OF DROSOPHILA[1]

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THE difficulty in obtaining rearrangements by treatment of Drosophila females has long been recognized (GLASS 1940, 1955). This difficulty might result from a relatively large volume of the oocyte nucleus causing breaks in non-homologous chromosomes to be more widely separated than in sperm. Furthermore, meiotic segregation would limit the recovery of those translocations induced. The techniques which have been employed may have been improper for the detection of the types of rearrangements which are possible in female germ cells. Finally, due to the considerable differences in sensitivity of oocytes in different stages (PATTERSON, BREWSTER and WINCHESTER 1932; PARKER 1955; KING, DARROW and KAYE 1957) the dosages used may have been high enough to guarantee nearly complete killing of the more sensitive cells which might give rise to a greater variety of rearrangements.

The technique of detachment of attached-X chromosomes has given a means for the study of translocation processes in Drosophila females. Though the frequencies obtained are relatively low when compared to translocation rates in male germ cells, rearrangements may be detected in the F_1, which makes possible the obtaining of as large numbers as one wishes.

The recovery of a detachment requires breakage of the attached-X in the proximal region, giving rise to separated X's, one having and the other lacking the centromere. Another break must occur near the tip of an X, Y, or autosome. Two products may result: the "centric X" with the tip of the other broken chromosome joining its broken end, or the "acentric X" which must join the broken end of the centric fragment of another chromosome to be recovered.

Detachments have been induced in newly emerged females and in those aged three or more days. Using the designations of KING, RUBINSON and SMITH (1956), these are stage 7 and stage 14 oocytes, respectively. PARKER (1954a,b) showed by means of fertility tests and tests for linkage of X and fourth chromosomes that nearly all detachments induced in stage 7 are translocations either with Y or with chromosome 4. The increased sensitivity of stage 14 oocytes, found in females three or more days old, together with the differences from stage 7 in sex ratio and phenotype of flies carrying a rearrangement, show that rearrangements induced in these older cells differ qualitatively as well as quantitatively from

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those in younger cells (PARKER and HAMMOND 1957, 1958). There must be a high frequency of induced lethality in this stage, since there is relatively poor recovery of males carrying detached X chromosomes. This may result from changes in the types of rearrangements being induced in aged cells. The work here reported deals with the identification of rearrangements picked up in the experiments of PARKER and HAMMOND as well as in some earlier ones, showing some of the differences in types of rearrangements induced in the two stages treated. An attempt is made to draw conclusions as to the types of exchanges which occur, based on a comparison of the frequency of recovery of complementary exchange products.

EXPERIMENTAL PROCEDURE

All of the detachments studied here were of the attached-X chromosome marked with *y*^{s} *su-w*^{a} *w*^{a} *bb*. In earlier work, various Y chromosomes had been used in the irradiated females to see any possible effect of the Y chromosome on detachment frequency (PARKER 1954a,b). PARKER and HAMMOND (1958) used the *sc*^{s} Y, since the yellow^{+} marker on the long arm of the Y gives a method for recognition of some X-Y translocations. In addition, the segregation of this marked Y might allow some inferences as to the translocation product recovered.

The testing procedure consisted of the following steps, in the order listed: (1) scoring phenotype and sex of the F_1 of treated females, (2) breeding of F_1 detachment females from some irradiations to see if males carrying the detached X chromosome were viable, (3) testing for X-4 translocations of all detachments viable in the male, (4) testing for male fertility to show attachment of a Y arm to the detached X, (5) testing for gross deletion of most of an X chromosome resulting in a "duplication" of the left tip of X, (6) testing for involvement of chromosome 2.

Tests for X-4 translocations were initiated in the original cross, where the irradiated attached-X females were mated to *Muller-5; ci ey*^{R} males. Males carrying a detached X were then crossed to attached-X females, *y; ci ey*^{R}. If the euchromatic part of 4 is linked with X, in the F_2 all males will be of *ci*$^{+}$ *ey*$^{+}$ phenotype; their attached-X sisters would usually be expected to be divided between *ci ey* and *ci*$^{+}$ *ey*$^{+}$ since usually we expect the male being tested to be hyperploid, and therefore to carry a free fourth chromosome with the normal alleles of the markers being used. Recovery of the acentric detached X chromosome with the centromere of chromosome 4, or appropriate nondisjunction of fourth chromosomes when the centric X is recovered with linkage of chromosome 4, may give rise to individuals which are not hyperploid. In this case, there will be only the *ci ey* class of attached-X females.

Where detachments recovered in females were being tested for X-4 translocations, females carrying a detached X balanced by Muller-5 were backcrossed to *Muller-5; ci ey*^{R} males. In most cases it was possible to recognize the X-4 translocation without further tests, but recognition of the presence of a free fourth carrying the wild type alleles of *ci* and *ey* was difficult as it required distinguish-

ing between heterozygous and homozygous Bar in eyeless flies. For this reason, stock cultures were established by crossing the appropriate males to *y; ci ey^R* females.

Male fertility in *Drosophila melanogaster* requires presence of both Y chromosome arms. It is possible to test for the presence of Y arms attached to the X chromosome by testing the fertility of males carrying the detached X and Y chromosome fragment having the fertility region of only one arm of the Y. The Y chromosome fragments used were marked with yellow$^+$ enabling one to follow their segregation and to eliminate the possibility of any tested males having a complete Y as a result of nondisjunction. Some types of detachments, e.g. X-4 translocations, give fairly high (ten percent) frequencies of primary nondisjunction of the Y, making it extremely likely that one would get false positive fertility tests, were this procedure not used. The presence of nondisjunctional individuals in the progeny of the tested males required repetition of the tests, using individual males, rather than mass matings. Detachments were recorded as having a given Y arm attached to the X chromosome only if the fertile males produced the expected regular classes with no nondisjunctional offspring. Males carrying the detached X to be tested were crossed to attached-X females which carried either the *sc^{VL}·*Y^S as the short arm of the Y, or *sc·*Y^L or *sc^s·*Y^L "CY-9" (LINDSLEY 1955) as the long arm of the Y. The F_1 males were then crossed to yellow attached-X females to see if they were fertile.

Gross deletion of most of one of the X's would give rise to an apparent detachment of the attached-X. This would leave the centric X with a small duplication of the tip of XL. Tests for this duplication were made by examining the crossover products of females heterozygous for the detached X and another X carrying yellow^1 and a duplication yellow$^+$ to the right of the centromere. Since deletional "detachments" would have yellow^2 both in the normal position and in the duplicated section, crossovers must be either yellow$^+$ or yellow^2. Presence of the yellow^1 crossovers is evidence of no X duplication including the yellow locus.

Detectable translocations of attached-X with major autosomes must involve breakage near the tips of the latter; otherwise the segregation of X chromosomes which would allow detection of the rearrangement would also create severe aneuploidy which would be lethal. Chromosome 2 was chosen for these tests because of the availability of good stocks for testing. The two products of this type of translocation would be the centric X joined with the tip of one arm of chromosome 2 (hyperploid for tip of 2L or 2R), or the acentric X joined to the broken end of one arm of chromosome 2 (hypoploid for tip of 2L or 2R). Males carrying detachments unidentified in previous steps were crossed to attached-X females carrying four recessive markers on chromosome 2, *y; al b c sp^2*. The male offspring were backcrossed to females like their mothers. A small duplication of a tip of chromosome 2 including *al$^+$* or *sp$^+$* would be recognized by production of F_2 males showing three of the four recessives as well as those being normal for all four, while their sisters would either show all four recessives or none. The attachment of an entire X near the tip of chromosome 2 might be recognized by deficiency of one of

the end markers, the F_1 males showing this one recessive. In the F_2, all males should be similar to the F_1 males and their attached-X sisters should be homozygous for the four recessives.

RESULTS

Table 1 summarizes the results of all tests described below.

Types of rearrangements produced in stage 7 oocytes

PARKER (1954a,b) has reported on some types of rearrangements which resulted in detachment of attached-X chromosomes. These results are included here, together with additional tests on the stocks which had not been identified at that time. In the case of detachments induced in the presence of Y fragments, tests for X-4 translocations and fertility tests have been carried out. With the $sc^s \cdot Y$ and the no-Y series, all of the appropriate tests described above have been carried out.

Detachments in no-Y females. It was reported previously that 61 out of 102 detachments induced in the absence of a Y were X-4 translocations. Tests of the remaining 41 failed to show any case of X deletion. Three cases of involvement of chromosome 2 were found, with two being hyperploid for 2L (al^+) and one hyperploid for 2R (sp^+).

Detachments with Y^S. Y^S #2. Tests for X-4 translocations showed nine such rearrangements out of 100 tested chromosomes. Of 23 of the remaining 90 tested, 18 were found fertile and were therefore $X \cdot Y^s$, while five were sterile in combination with $sc \cdot Y^L$.

Detachments with $sc^{v_1} \cdot Y^S$. The acrocentric Y-short, $sc^{v_1} \cdot Y^s$, gave a ratio of 68 detached X chromosome carrying the yellow$^+$ locus to 42 which did not. Fifty of the former and 40 of the latter were tested for X-4 translocations and of these, eight of yellow^2 phenotype were found to have X and 4 linked. Fertility tests were run on 28 of the 32 not found to have X-4 translocations; 20 were found to be $X \cdot Y^s$. Forty of the 50 yellow$^+$ stocks were tested for linkage of Y-short, and all proved to be sterile in combination with $sc \cdot Y^L$.

Detachments with $sc^{s_1} \cdot Y^L$ #2. This is a nearly rod-shaped Y-long arising by inverted exchange with the distal end of the Muller-5 chromosome. Thirty-eight detachments, 22 of which were yellow^2 and 16 yellow$^+$, were recovered. None of the yellow$^+$ group had Y-long linked with the X, while two of the yellow^2 group where $X \cdot Y^L$, two were X-4, and 12 were unidentifiable by these two tests. The remainder were not tested.

Detachments with $sc \cdot Y^L$. In this case, of 67 detachments, 43 were yellow^2 and 24 yellow$^+$. Ten of the yellow^2 class were X-4 translocations; 20 of the remainder were tested and three were found to be $X \cdot Y^L$. Nineteen of the yellow$^+$ were tested for Y-linkage, and all were found to be sterile in combination with $sc^{v_1} \cdot Y^s$.

Detachments with $sc^s \cdot Y$. PARKER (1954a) tested 68 detachments induced in females carrying $sc^s \cdot Y$ and found the following. There were 30 cases where yellow$^+$ was linked with the X; nine of these proved to be $X \cdot Y^L$ while the remaining 21 were sterile in combination with either the Y-long or Y-short fragment. Thirty-eight were of yellow^2 phenotype; nine proved to be X-4 translocations, 19

TABLE 1

Estimated proportions of various types of rearrangements involved in detachment of attached-X chromosomes

Y Chromosome present	Stage treated	Rearrangement Types: Translocations with Y: $X{\cdot}y^+$	$XY^L{\cdot}Y^S$	$X{\cdot}Y^L y^+$	$X{\cdot}Y^S$	$X{\cdot}Y^L$	$XY^S{\cdot}Y^L y^+$	X Deletions	Translocations with autosomes: X-4	2L Hyperploids	2R Hyperploids	2L or 2R Hypoploids
$sc^8{\cdot}Y$	7*	.31	.06	.13	.28	0	0	0	.13	0	0	0
$sc^8{\cdot}Y$	14	.16	.03	.02	.16	0	.02	.06	.10	.09	.03	.04
no Y	7*	..	..	..	..	..	..	0	.60	.02	.01	0
$Y^S{\cdot}Y^S$ # 2	7	..	..	..	.70	..	..	..	.09	..	..	..
$sc^{V1}{\cdot}Y^S$	7	.61	..	..	.22	..	..	..	.08	..	..	..
$sc^{S1}{\cdot}Y^L$ # 2	7	.42	..	0	..	.08	..	..	.08	..	..	..
$sc{\cdot}Y^L$	7	.36	..	0	..	.07	..	..	.15	..	..	..

* Data on stage 7 with $sc^8{\cdot}Y$ or with no Y from PARKER (1954a), except for tests for X-deletion and chromosome 2 involvement, which are from current data.

were X·Y^S, while four were XY^L·Y^S as the males were fertile in the absence of a free Y. The remaining six were not accounted for by the preceding tests.

Subsequently, tests have been run on these six. No cases of gross deletion of the X, nor of exchange with chromosome 2 were found. We can account for about 90 percent of detachments as being products of translocation between X and Y or X and fourth chromosomes.

Recent experiments have given a large number of detachments which were not analyzed, except to record linkage of yellow$^+$ and linkage of X and fourth chromosomes. Of 1706 detachments obtained, 976 were recovered in females, 393 were yellow^2 males and 337 yellow$^+$ males. Two hundred and six of the yellow^2 stocks were tested for X-4 translocations, and 30 cases of this type of rearrangement were found, which makes about eight percent of all male-viable detachments this kind of rearrangement.

Types of rearrangements induced in stage 14 oocytes

All detachments studied here had been obtained by irradiation of aged females carrying sc^s·Y. Of 2116 detachments induced in stage 14 oocytes, 1449 were recovered in females, 553 were yellow^2 males, and 114 were yellow$^+$ males. In a random group of 391 stocks which were male-viable and recovered in either sex, there was a ratio of 315 yellow^2 to 76 yellow$^+$ stocks, i.e., about 20 percent carried at least a part of Y-long from sc^s·Y.

Three hundred and seventy-three of the yellow^2 stocks were tested for X-4 translocations, and 48 such cases were found. This means about ten percent of all male-viable detachments are X-4 translocations.

A group of 138 detachments, all negative for X-4 translocations, were chosen for analysis. Thirty-three which carried yellow$^+$ were tested with both Y arms for male fertility; three proved to be X·Y^L, while three were fertile with no free Y, hence were probably XY^S·$Y^L sc^s$. Otherwise, there must have been some reorganization of the sc^s·Y in these three cases so that the sc^s piece was no longer on the tip of Y-long, as in the case of sc^s·Y:bw^+, as shown by BAKER (1955). The remaining 27 were sterile in combination with either Y-short or Y-long fragments.

One hundred and five of the yellow^2 stocks were tested with both Y fragments for fertility. Twenty-three were found to be X·Y^S, and four were XY^L·Y^S since these gave fertile males with no added Y fragment. This makes about 19 percent of all detachments X-Y translocations of these two types.

The exchanges between chromosome X and 4 or Y, which accounted for 90 percent of all detachments in stage 7 can account for about 50 percent in stage 14. The great excess of yellow^2 males suggested the occurrence of types of rearrangement where the preferred detachment (the centric X) would segregate from the sc^s·Y at the first meiotic division, hence no free sc^s·Y would be recovered. This would mean recovery of a centric X with a duplication of the tip of an X or of an autosome.

Seventy-four of the 77 yellow^2 stocks which were negative in fertility tests were tested for duplication of the tip of X; nine such cases were found, with a duplica-

tion carrying the mutant, y^2. These account for about six percent of all detachments.

Sixty-three of the remaining 65 stocks were tested for involvement of chromosome 2; 12 had duplications of 2L (al^+) linked with X, four had 2R (sp^+) linked with X, and five had the entire second chromosome linked, including normal alleles of all of the four markers used. In addition, these flies are not Minute, hence they are not deficient for the *M(2)33a* locus. The break in chromosome 2 in each case must lie outside the genetic map. ABRAHAMSON, HERSKOWITZ and MULLER (1956), who recovered one case of this same type, likewise did not find it deficient for any known locus on chromosome 2. Identifiable exchanges with chromosome 2 are found to account for about 16 percent of all recovered male-viable detachments.

Without testing for ends of chromosomes 3 or one end of chromosome 4, we are able to account for over 70 percent of all detachments as being translocations or deletions. It is likely that there are also exchange products involving breaks in the chromosomes distal to the markers being used in these tests, so that hyperploidy is not detectable. The indirect evidence for a significant number of these will be discussed below.

Non randomness of recovery of complementary exchange products

One of the questions of significance is the nature of the exchanges induced: that is, do these involve chromatids or whole chromosomes? There are two lines of evidence on this. In experiments with individual females, it is possible to recover a detached X with part of the Y chromosome linked to an X, and to recover an entire Y chromosome at the same time, where the original treated female had only one Y chromosome. Two cases have been found in which an $X \cdot Y^S$ chromosome was recovered along with an unaltered Y; in one case the Y was $sc^8 \cdot Y$, in the other $sc^{V1} \cdot Y^S$. Both of these recovered Y's were subjected to male-fertility tests to show their completeness. In both cases, all of their attached-X sisters were yellow^2 as would be expected if the mother had only one Y chromosome.

Another basis for the conclusion that exchanges involve individual chromatids is the non randomness of recovery of complementary exchange products. In stage 7, detachment in the absence of the Y gives about 60 percent X-4 translocations; the complementary type, having the heterochromatic portion of chromosome 4 linked with X, can make up no more than 38 out of the 102 cases tested. In the $sc^8 \cdot Y$ series, there were 21 cases where only the yellow$^+$ was linked with X, while the complementary product, $XY^L \cdot Y^S$, was represented by four cases. The ratio of $X \cdot Y^L$:$X \cdot Y^S$ was 9:19. Similar figures are found in the case of each Y chromosome fragment used, where complementary types may be recognized. Detachments in stage 14 oocytes give similar figures. There were 23 $X \cdot Y^S$: 3 $X \cdot Y^L$ and 27 yellow$^+$ (without Y-long fertility): 4 $XY^L \cdot Y^S$. Since the sequence is $sc^8 \cdot Y$ is $y^+ Y^L$-$bb^+ Y^S$, breakage of this chromosome must be about equally divided between the long and short arms, with breakage in the short arm usually being proximal and in the long arm distal to fertility factors.

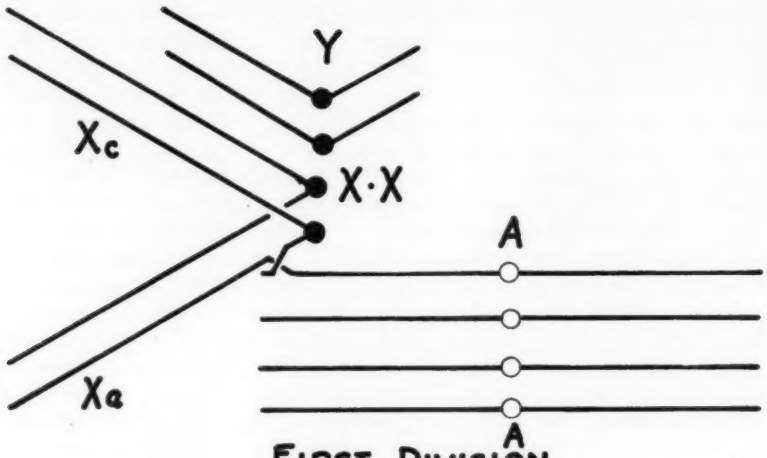
The basis for interpretation of this is the finding by NOVITSKI (1951) that there is non random segregation of unlike chromatids in the second meiotic division, with the shorter chromatid being more likely to be included in the egg nucleus. The cases studied at that time gave about a 2:1 recovery of the shorter chromatid. More recently, NOVITSKI and SANDLER (1956) have been able to extend the applicability of the principle of non randomness to include the tandem acrocentric compound X, where they found cofficients of non randomness as high as 0.92.

There was one difficulty in making the interpretation that non randomness implied chromatid exchanges. The results obtained with use of sc^{v1}·Y^s, where the majority of detachments carried the yellow$^+$ marker and about one third as many were X·Y^s, required the assumption of a high frequency of breakage in the Y between the centromere and yellow$^+$. Since this Y is derived by crossing over of a normal Y chromosome with the pericentric sc^{v1} inversion, this piece would be expected to be very small in relation to the short arm of the Y.

Irradiation of reversed acrocentric compound X's with sc^{v1}·Y^s gave a total of ten detachments, all the proximal (centric) X. In four of these cases, the yellow$^+$ was linked with the detached X; the remainder were not tested. Since the recovery of the centric X requires the recovery of the acentric fragment of the other broken chromosome, this shows a high breakage frequency between the centromere and yellow$^+$, as expected. The reversed acrocentric used was *y ac sc pn-*sc^s·, supplied by LINDSLEY. These irradiated females, also carrying sc^{v1}·Y^s, were crossed to yellow *Muller-5* males carrying the *y* sc^s· Y of LÜNING, thus allowing males with the detached proximal X deficient for the yellow and achaete loci to survive.

Chromatid translocations involving autosomes should allow recovery of the centric X more often than the acentric for another reason. Figure 1 shows the sixteen possible products of a primary oocyte in which such an exchange has occurred. It can be seen that the centric X should be detected in three and the acentric in only two of the sixteen products. In case of translocations with the Y chromosome, since the Y normally should segregate from the X at the first division, the difference in recovery of centric and acentric X's should depend primarily on non random second division segregation.

An alternative interpretation of non randomness has been suggested by ABRAHAMSON, HERSKOWITZ and MULLER (1956), where they conclude that the higher rate of recovery of "capping" than of "capturing" is due to the relative inviability of the two classes of aneuploids, with hyperploids being more viable than hypoploids. This argument might apply with more force in stage 14 than in stage 7 oocytes, and in the case of X-autosomal translocations than with X-Y translocations, where the hypoploidy of the X in many cases would be covered by the hyperploidy of a segement of the Y. In every case the hypoploid X would be recovered either with a complete X or a complete Y chromosome, and if breaks are confined to the proximal heterochromatin, there should be little effect on viability. However, there is non random recovery of complementary products with all testable types of rearrangement.



FIRST DIVISION

$\frac{X \cdot X}{X_c}$; $\frac{A}{X_a\text{-}A}$	$\frac{Y}{Y}$; $\frac{A}{A}$	$\frac{X \cdot X}{X_c}$; $\frac{A}{A}$	$\frac{Y}{Y}$; $\frac{A}{X_a\text{-}A}$

SECOND DIVISION; EGGS

X·X; A	X_c; A	Y; A	Y; A	X·X; A	X_c; A	Y; A	Y; A
* X·X; X_a-A	** X_c; X_a-A	Y; A	Y; A	X·X; A	X_c; A	Y; X_a-A	Y; X_a-A

FIGURE 1.—Diagram of a translocation between attached-X and an autosome, showing products of first and second divisions, to show inequality of chromatids which will separate at Anaphase II. A, autosome; Y, *sc*[8]·Y; X·X, attached X; X_c, "centric" X; X_a, "acentric" X; X_a-A, "acentric" X attached to tip of autosome.

* This product dies.

** This product cannot be distinguished from attached-X without further testing.

The argument of aneuploidy can hardly apply in the case of detachment of reversed acrocentric compound X's involving exchanges with a Y chromosome, as X hypoploidy should be as likely in the proximal (centric) as in the distal (acentric) X, since breaks anywhere in the interstitial heterochromatin (or perhaps even in adjacent euchromatic regions) may give rise to centric-X detachments. The number of cases of this type reported here is small, but in each of the ten cases the proximal X was the one recovered.

The interpretation of non randomness as the result of chromatid translocations means that where breaks occur to give rise to recoverable detachments, the centric X must be the one more often recovered. The centric X will rejoin with the acentric tip of another chromosome, so that the single X will be smaller than the complete attached-X it will separate from at the second division. The acentric X must be joined near the tip of another chromosome, Y or autosome, and this rearranged chromatid must therefore be longer than the normal chromatid it separates from at the second division. We may expect the coefficients of non randomness to vary considerably for the different types of exchange, and it would be difficult to estimate the frequencies of unidentifiable exchange products.

Another argument for the recovery of the centric X in most cases is the relatively infrequent recovery of the unaltered sc^8· Y along with the detached X. If normal first division segregation occurs, the centric X is separated from the unbroken sc^8· Y; the acentric X, joined to the centric part of another chromosome, can be recovered separately only if it separates from the centric X at the first division. Hence, recovery of the acentric X should also mean recovery of sc^8·Y unless nondisjunction occurs. There are some reasons for believing this may not always be the case.

The recovery of the centric X should require that the individual be hyperploid for the tip of the Y or autosome involved in the translocation, while the recovery of the acentric X implies a corresponding hypoploidy. In the case of a major autosome, chromosome 2, it is possible to distinguish between these two types, while with rearrangements involving Y or fourth chromosomes there are difficulties in distinguishing directly between the two products. Recovery of the centric X with 4 linked should always be in an individual hyperploid for the euchromatic part of chromosome 4: i. e., the egg should have a free fourth chromosome in addition to the four attached to X. Breakage of chromosome 4 in the heterochromatic arm likewise can give rise to a chromosome showing X-4 linkage. In this case, it would be expected to be recovered along with sc^8· Y. In stage 7 oocytes, out of 30 X-4 translocations, 26 carried two free fourth chromosomes, while four cases had only one. In stage 14, out of 48 X-4 translocations, 36 carried two free fourth chromosomes and 12 had only one. In only two cases, X-4 translocations induced in stage 7 oocytes were recovered with sc^8· Y, while none of those induced in stage 14 were recovered with the sc^8· Y.

Another difficulty lies in the failure to recover sc^8· Y in any of the five cases where the acentric X was linked to chromosome 2. One would have to assume a

high rate of nondisjunction of Y and centric X to account for the repeated failure to realize the expectation with this type of exchange.

We have been able to identify the chromosomes involved in detachments in stage 7 oocytes in about 90 percent of the cases, and in stage 14 oocytes in over 70 percent of the cases. The high frequency of involvement of chromosome 2 in the latter case might lead us to expect some increase in involvement of chromosome 3. However, there are several possibilities involving chromosomes tested for, which cannot be excluded. If breakage in the heterochromatic arm of chromosome 4 is high, non randomness would favor the recovery of the centric X with the unmarked chromosome 4 arm. This, too, could account for a significant number of the unidentified cases. The finding of five cases of linkage of X with the entire chromosome 2, with no evidence of breakage within the genetic map, i.e., between aristaless and Minute (2) 33a, means that the complementary type, hyperploid for the unmarked tip of chromosome 2 which has joined the centric X, could not be detected by these tests. This product should be greatly favored in the second division because of the great inequalities in sizes of separating elements. These five cases of apparent translocation of an entire X to the end of the entire second chromosome are of the kind which WHARTON (1943) found to have occurred in the case of three Drosophila species: *spinofemora*, *testacea*, and *tranquilla*.

Lethality

The problem of the origin of lethality in detachments of attached X has been discussed (PARKER 1953, 1954a) and the conclusion reached that recessive lethals may accumulate in the proximal region of attached-X chromosome, and perhaps ultimately become balanced by other very closely linked spontaneous lethals. The basis for this interpretation is the difference in behavior of several different attached-X lines. There is very little induced lethality resulting from rearrangement. These data were obtained by the irradiation of stage 7 oocytes; when stage 14 cells are treated, a different situation is found. This difference may be seen in the various tables of PARKER and HAMMOND (1958), where relatively about twice as many females per male carrying the detachments are found resulting from treatment of stage 14 as compared to stage 7. In the stage 7 results, there is a ratio of 976 females to 730 males in the detachment classes, giving 1.34 females per male. In stage 14, there were 1449 females to 667 males, or 2.17 females per male.

Tests for viability of males carrying the detached X recovered in females were carried out on 267 females derived from treated stage 14 oocytes; 65 were sterile (2^4 percent), and 68 of the remaining 202 X chromosomes were lethal in the male (34 percent of fertile females). When stage 7 cells were treated, a total of nine out of 110 tested chromosomes were found to be lethal in the male (eight percent of fertile females).

The difference in sex ratio may be accounted for by the increase in induced lethality. It is evident that many changes are sublethal, as many of the females derived from treated stage 14 show very low fertility and poor recovery of males

carrying the detached X. The high frequency of sterility, low fertility, and the general appearance of the detachment females recovered suggest aneuploidy.

That the increased lethality is due to the rearrangement and not to independently induced recessive lethals is shown by data obtained by irradiation of Oregon-R females of similar ages with similar doses. Oregon-R females were irradiated in each stage, and lethal rates measured by the Muller-5 method. Stage 7 oocytes given 2000r of X-ray gave an F_1 ratio of 1298 females to 1346 males. Tests were made of 1209 F_1 females; 20 were sterile, and 39 of the remaining 1189 chromosomes were lethal in the male. When stage 14 was treated with 500r of X-ray, the F_1 count gave 1437 females and 1398 males. Tests of 1129 F_1 females showed 20 to be sterile, and 13 of the remaining 1109 chromosomes were lethal in the male. The types of changes which may be induced in the free X chromosome cannot account for the induced lethality in the detachments. The greater variety of rearrangements possible in stage 14 has already been seen. Perhaps a higher frequency of types of rearrangement which can give rise to more severe aneuploidy is the basis for the increased frequency of lethal detachments in stage 14 oocytes.

GENERAL DISCUSSION

Various interpretations have been advanced to account for differences in response to irradiation of cells of different types. KAUFMANN and WILSON (1949) suggest that failure of breaks to rejoin in Drosophila sperm until after sperm entrance is due to restriction of movement in the sperm nucleus. CROUSE (1950) similarly accounts for failure to obtain translocations by irradiation of *Sciara coprophila* oocytes as due to the "failure of non-homologous chromosomes to contact each other before restitution or intra-chromosomal rearrangement has occurred."

GLASS (1955) has pointed out some difficulties in making comparisons of relative frequencies of different types of rearrangements in oocytes and in mature sperm of Drosophila, particularly in the case of complete recovery of a chromatid translocation in oocytes. In addition to this difference, one should recognize the effect of non random disjunction on the recovery of each of the kinds of aberrations. If the exchanged segments in translocations are grossly unequal, one might conceivably lower the probability of recovery of the complete translocation to less than one percent. Deficiencies induced in females should be favored in recovery while inversions presumably would behave as normal sized chromosomes. Some inversions would be lost through anaphase bridge formation if appropriate chiasmata occurred, but a similar number of cases might be expected where potential radiation-induced bridges would be resolved by the same chiasma configuration. In the absence of sister-strand crossing over, breaks at different loci in two sister chromatids could give rise to deficiency (or duplication) but not to inversion.

WHITING (1945) has interpreted the differences in sensitivity of different meiotic stages in the Habrobracon oocyte in terms of the condition of the chromosomes: "Tension is the main factor in determining the permanence of breaks

caused by ionizations, chromosome form and movement in determining the nature of the new combinations of broken ends." BOZEMAN and METZ (1949) correlate the change in senstivity in *Sciara ocellaris* oocytes with the breakdown of the nuclear membrane, postulating intervention of a cytoplasmic agent which is radiation-induced or which sensitizes the chromosomes to radiation. They suggest alternatively that some healing process ("formation of matrix material") may intervene in the less sensitive stages before rearrangement may occur.

It is possible to distinguish at least three levels of sensitivity in the Drosophila oocyte. PARKER (1953, 1954a) was able to show that detachments of attached-X chromosomes were obtained almost exclusively from the oldest 60–90 oocytes in newly emerged females. PARKER (1955) and PARKER and HAMMOND (1957, 1958) obtained a still higher level of sensitivity by aging females three or more days before treatment, and suggested the possibility of differences in oxidative metabolism as a basis for differences in sensitivity of stages 7 and 14 oocytes. If this is true, then failure of rejoining of many breaks in mature sperm might be due to nonavailability of energy required for rejoining rather than limitations of space or distribution within the sperm nucleus. SPARROW and MALDAWER (1950) found a difference in ratio of acentric fragments to dicentrics and rings in Metaphase I and postmeiotic interphase cells in Trillium, and concluded that the more sensitive Metaphase I cells showed less rejoining of breaks, but concluded that differences in sensitivity must in part be due to differences in the number of primary breaks induced. KING, DARROW and KAYE (1956) likewise can account for at least three levels of sensitivity in the Drosophila oocyte. KING (1957) accounts for these differences in part at least by differences in diffuseness of the chromatin.

Table 1 shows the considerable difference between stages 7 and 14 in kinds of rearrangements found. On the basis of the evidence available it is not possible to account for the difference in behavior of the two stages. As has been suggested by various authors, the changes could be due to changes in nuclear structure, with greater coiling and with closer association of non-homologous chromosomes in stage 14; this would be consistent with the description given by KING, RUBINSON and SMITH (1956). This would account for the increase both in X deletions and in autosomal translocations, relative to X-Y translocations. BOZEMAN and METZ (1949) found comparable changes in types of rearrangements in Sciara. On the other hand, since breaks rejoin more slowly in stage 14, it is possible that restitution is much less likely than in stage 7, and much more widely separated breaks are able to join together to give a greater variety of rearrangements.

These results may also suggest a reason for the difficulty in inducing interchromosomal rearrangements heretofore. Stage 7 oocytes give very few detachments involving the major autosomes, and it seems unlikely that many translocations between two major autosomes or between a free X and a major autosome could be induced. Stage 14 would be much more likely to give recoverable translocations by the usual procedures. However, the dosages most probably employed would guarantee recovery almost exclusively of stage 7 products. At a dose of

about 2500r, 75 percent of stage 7 would survive, while less than 1.5 percent of stage 14 oocytes would remain viable (PARKER 1955; SONNENBLICK 1940). It is possible that a dose of about 500r given to stage 14 might give rise to a variety of rearrangements which would be recoverable with suitable screening methods, such as the bithorax method of LEWIS (1954).

SUMMARY

Tests were made on a number of detachments of attached-X chromosomes induced either in young females (stage 7 oocytes) or aged females (stage 14 oocytes). These were tested for translocations with Y, second, or fourth chromosomes and for gross deletion of an X chromosome. It is possible to account for about 90 percent of detachments in stage 7 and 70 percent in stage 14 by these types of rearrangement. Aging results in an increase in frequency of autosomal translocations relative to X-Y translocations, with the relative increase in involvement of chromosome 2 being greater than in chromosome 4. There is also a significant number of X deletions in stage 14, while none were found in stage 7.

It is concluded that the rearrangements involve individual chromatids, since complementary translocation products are recovered in unequal numbers. It is suggested that a large number of these rearrangements may be hyperploid for small unmarked tips of the various chromosomes involved since one class of rearrangement involved breakage of chromosome 2 distal to the terminal markers.

The increased lethality of detachments induced in stage 14 is attributed to the increased variety of rearrangements involved, with greater possibility of more severe aneuploidy. The induced lethality cannot be accounted for by the independent induction of recessive lethals or sublethals in the detached X.

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STUDIES WITH PURPLE ADENINE MUTANTS IN NEUROSPORA CRASSA. III. REVERSION OF X-RAY-INDUCED MUTANTS[1, 2]

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INTEREST in the problem of reversibility of X-ray-induced mutations has been renewed in recent years, owing to results from a number of experiments on selected loci in Drosophila and maize that conflict with results obtained by earlier investigators. Experiments of PATTERSON and MULLER (1930) and TIMOFÉEFF-RESSOVSKY (1932) demonstrated that X-rays can cause reversion of either spontaneous or X-ray-induced recessive mutations at a number of different loci in Drosophila. Their results seemed consistent with the hypothesis that X-ray-induced and spontaneous gene mutations are essentially similar in nature and are caused by intragenic alteration.

Evidence accumulated by STADLER (1941) in maize, however, suggested clear differences between X-ray-induced and spontaneous mutation. A study of the effects of X-irradiation on the *A1* locus (STADLER 1944; STADLER and ROMAN 1948) indicated that X-ray-induced mutations were caused largely, or wholly, by such extragenic alterations as deletions, duplications, or chromosome rearrangements, which only simulate true gene mutation.

LEFEVRE (1950) attempted to induce reverse mutation with X-rays in both germinal and somatic tissue of Drosophila and got the same negative results as STADLER in his experiments with maize. Since LEFEVRE was unable to obtain evidence for reverse mutation, he questioned the reliability of the early reports of X-ray-induced reverse mutation in Drosophila, and concluded that X-ray-induced mutations in Drosophila, as in maize, are not qualitatively similar to spontaneous mutations and that X-radiation is principally, or perhaps wholly, destructive and results in gene loss in both organisms.

GILES and his collaborators (1951, 1955), however, showed that X-rays *can* induce mutations of positive dominant action at the inositol and purple adenine loci in *Neurospora crassa*. Both these papers present evidence that after X-irradiation, reverse mutations were obtained in which normal adenine or inositol syn-

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[2] During the major portion of this investigation the author held a predoctoral fellowship from the National Cancer Institute of the National Institutes of Health, Public Health Service.

[3] A portion of this investigation was performed and the report was prepared at Oak Ridge National Laboratory, operated by Union Carbide Nuclear Company for the U.S. Atomic Energy Commission.

thesis, interrupted by the original mutation, was restored as a result of genetic changes localized at the purple adenine or inositol loci. However, the mutants used for these studies were induced by ultraviolet radiation or nitrogen mustard, and there still remained some question about the reversibility of X-ray-induced mutants.

The main purpose of this study was to obtain X-ray-induced purple adenine mutants in Neurospora, and to investigate their mutability. If all X-ray-induced mutations that simulate true gene mutations are actually the result of such extragenic alterations as deletions, these changes should be genetically stable. If, however, some X-ray-induced changes result from gene mutation, they should be capable of subsequent change either spontaneously or with further X-irradiation.

The "filtration-concentration" technique of WOODWARD, DE ZEEUW, and SRB (1952, 1954) was used to obtain a large number of purple adenine mutants both from nonirradiated and X-irradiated conidia of a single wild type strain. Initially it was thought that such mutants represented a series of alleles at the *ad-3* locus (MITCHELL and HOULAHAN 1946; BARRATT *et al.* 1954). Subsequent heterokaryon, crossing, and tetrad analyses, however, showed two separate but closely linked loci in the *ad-3* region, which have been designated *ad-3A* and *ad-3B* (DE SERRES 1956; GILES, DE SERRES and BARBOUR 1957). In this paper, evidence about the origin, linkage relations, and mutability of purple adenine mutants at both these loci is presented in detail.

MATERIALS AND METHODS

The wild type strains used, 74A and 73a, derived by morphological and cytological selection of progeny from crosses of Emerson's strains E-5256A and E-5297a, were obtained from DR. PATRICIA ST. LAWRENCE, of Yale University. The mutant strains used are as follows: *hist*-2 (C94) histidine (HAAS *et al.* 1952) and *nic-2* (43002) nicotinic acid (BEADLE and TATUM 1945).

In general, the media used were those described by BEADLE and TATUM (*op. cit.*). For crosses, synthetic crossing medium (WESTERGAARD and MITCHELL 1947) was used with the appropriate biochemical supplementation. Minimal sorbose agar, which has been used throughout these experiments, consists of 0.1 percent sucrose and 0.8–1.0 percent sorbose to inhibit colony size (TATUM, BARRATT and CUTTER 1949). Random ascospore analyses were made by the overplating technique developed by NEWMEYER (1954).

RESULTS AND CONCLUSIONS

The origin of purple adenine mutants obtained from filtration-concentration experiments

The "filtration-concentration" technique of WOODWARD, DE ZEEUW and SRB (1952, 1954) allows screening for mutant conidia of a particular biochemical type in a population of nonmutant conidia, since only nonmutant conidia will germinate and grow in a minimal medium. When the mycelium formed by the

nonmutant conidia is removed, the mutant conidia can be concentrated and specific mutants obtained by selective plating techniques. When macroconidial strains are used in these experiments, not all of the mutant nuclei present in the total conidial population are recoverable since multinucleate conidia containing viable wild type nuclei germinate and grow in minimal medium and mutant nuclei in such conidia are thus lost. Because of this, the mutants recovered from filtration experiments on untreated macroconidia are those derived solely, or almost entirely, from that proportion of viable uninucleate conidia present initially in the total conidial population. However, the effectively uninucleate proportion of a macroconidial population can be increased artificially with X-irradiation, by inactivation of nuclei in multinucleate conidia (ATWOOD 1952; ATWOOD and MUKAI 1954). Experiments to estimate the nuclear distribution in macroconidia from strains grown on minimal agar have shown that the viable uninucleate proportion for untreated conidia is about 20 percent, whereas, after X-irradiation at 50 percent survival this proportion increases to about 78 percent of the total surviving conidia (ATWOOD, personal communication). Thus the total viable uninucleate population in aliquots of a conidial suspension would differ by a factor of two, depending on whether the aliquots were untreated or were X-irradiated to give 50 percent survival. Because of this effect, it seemed possible that the increased recovery of purple adenine mutants from X-irradiated macroconidia could be caused, at least in part, by filtration and concentration of pre-existing mutants of spontaneous origin in the increased uninucleate population resulting from X-irradiation.

To test this possibility, reconstruction filtration experiments were performed to determine whether pre-existing purple adenine mutants were recovered from X-irradiated macroconidia subjected to the filtration-concentration procedure in proportion to the increase in the total uninucleate population. Such experiments were performed on heterokaryons with disproportionate mutant: wild type nuclear ratios of about 1:3000 and 1:30,000 to mimic the distribution of mutant nuclei of spontaneous origin in macrocondia. Such heterokaryons were made according to the techniques developed by ATWOOD and PITTENGER (1955) and PITTENGER, KIMBALL and ATWOOD (1955) using conidia from a genetically marked *ad-3A ad-3B* double mutant strain and a genetically marked adenine-independent strain. Heterokaryons with previously established nuclear ratios were grown on complete medium to increase the average nuclear number and to decrease the proportion of uninucleate conidia (HUEBSCHMAN 1952) in the untreated conidial population. Under these conditions, the influence of the production of effectively uninucleate conidia by irradiation is maximized.

The number of purple adenine mutants recovered by direct plating of X-irradiated conidia from these heterokaryons is considerably greater than the number recovered from untreated conidia, and this is accounted for by the increase in the number of effectively uninucleate conidia. However, if the untreated and X-irradiated conidia from these heterokaryons are subjected instead to the filtration-concentration procedure, pre-existing mutants are not recovered in pro-

portion to the increase in the effectively uninucleate population. Moreover, the evidence indicates that fewer pre-existing mutants are recovered from X-irradiated conidia than untreated conidia. In Table 1, the results of reconstruction

TABLE 1

The recovery of pre-existing purple adenine mutants after X-irradiation of macroconidia from heterokaryons with disproportionate nuclear ratios using the filtration-concentration technique

Nuclear ratio wild type:mutant	X-ray dose ($r \times 10^3$)	Total viable conidia incubated ($\times 10^7$)	Percentage survival	Total mutants recovered per ml of filtrate	Percentage recovery
3,000:1	None	18.7	...	43.5	...
	36	11.9	63.6	29.0	66.6
30,000:1	None	22.5	...	2.8	...
	36	14.9	66.2	1.8	64.3

filtration experiments on untreated and X-irradiated aliquots of condial suspensions from two different heterokaryons, show that the recovery of pre-existing mutants from X-irradiated conidia is about 65 percent of the recovery from untreated conidia, where a dose of 36,000r gave a survival of 65 percent for the total conidial population. These results suggest that there is an interaction between viable mutant nuclei and inactivated wild type nuclei in multinucleate conidia sufficient to give limited growth of such conidia incubated in minimal medium. Thus, during the incubation period in minimal medium, such conidia that would produce mutant colonies by direct plating are filtered off along with conidia containing only wild type nuclei and are not recovered. Hence it appears that the filtration-concentration technique provides an adequate measure of the incidence of induced mutations without interference from pre-existing spontaneous mutations, unless the incidence of spontaneous mutations is high.

Origin and numbers of individual mutant strains

A large number of purple adenine mutants derived from X-irradiated conidia were obtained by the filtration-concentration technique. The incidence of spontaneous purple adenine mutants was determined by comparable filtrations with nonirradiated conidia. Sampling from the latter filtrations yielded two mutants, both of which were *ad-3A*. Filtration and equivalent sampling of conidia irradiated with doses of 10, 20, 35, and 200×10^3r yielded eight *ad*-3A mutants and 42 *ad-3B* mutants.

The formulations of STEVENS (1942) were used for determinations of the expected number of spontaneous purple adenine mutants derived from X-irradiated conidia. No *ad-3B* mutants were recovered from untreated conidia; the range in

number of mutants expected (P=0.05) is therefore 0–3.69. Since fewer pre-existing mutants are expected from irradiated than from nonirradiated conidia, two or three of the 42 *ad-3B* mutants may have originated spontaneously. Two *ad-3A* mutants were recovered from untreated conidia; hence the range in expected number of mutants (P=0.05) is 0.242–7.22. Similarly, four or five of the 8 *ad-3A* mutants recovered may have been of spontaneous origin.

The first 24 purple adenine mutants (three *ad-3A* and 21 *ad-3B*) recovered in the filtration-concentration experiments were used in this study; their origin and designation are presented in Table 2. The system of terminology used in preliminary reports (GILES, DE SERRES and PARTRIDGE 1955; GILES 1956) of these experiments was changed to a system that gives the locus designation used in the present series of papers.

TABLE 2

Origin and mutant numbers of purple adenine mutants derived from wild type strain 74A

Experiment number	X-ray dose ($r \times 10^3$)	Locus designation: Present		Locus designation: Previous*	
		Ad-3A	*Ad-3B*	*Ad-3A*	*Ad-3B*
Y59	200	..	B1	...	M1
Y68	200	A1	....	M2	....
Y83	35	..	B2-B8	...	M3-M9
Y112	none	A2	....	M21	....
	35	A3	B9-B21	M23	M10-M20, M22,M24

* GILES 1956.

Growth characteristics of the mutants

Qualitative biochemical tests on the 24 strains showed that all the mutants except B16 and B21 have the same general characteristics as pre-existing purple adenine mutants: (1) no growth on minimal medium, (2) more intense purple pigment formation at 35° than at 25°C, and (3), under the longer wave lengths of ultraviolet light, more intense blue fluorescence of filtrates of cultures grown at 35° than at 25°C. At the latter temperature, mutants B16 and B21 show only partial requirements for adenine, but B16 grows on minimal medium only after prolonged incubation. At 35°C, both mutants exhibit a more extreme mutant phenotype, B16 grows only on supplemented minimal medium and B21 shows only limited growth on minimal medium. Filtrates of both mutants, grown at 25° or 35° C, fluoresce blue; but at 25°, purple pigment is formed only on minimal medium. Supplementation with 50 μg/ml of either adenine or hypoxanthine prevents any detectable pigment accumulation.

GENETIC ANALYSIS

Effect of individual purple adenine mutant strains on crossing over in the hist-2—nic-2 *interval*

To determine whether any of the purple adenine mutants had resulted from genetic alteration restricted to the *ad-3* region, we first had to find out whether the linkage relations (with closely linked markers) of the individual mutants were different from "standard" or "normal values" for these same intervals. Previous studies showed that the *ad-3* region is located between the closely linked *hist-2* and *nic-2* loci, and that *ad-3A* and *ad-3B* loci are probably less than 0.15 crossover unit apart (DE SERRES 1956). Since it seemed reasonable to assume that a spontaneous mutant was less likely to have resulted from extragenic alteration than any of the X-ray-induced mutants, the recombination frequencies obtained for the *hist-2—ad-3* and *ad-3—nic-2* regions with such a mutant were used as "standard values" for these regions.

Colony counts were used for estimating the number of adenine-independent segregants in parental and recombinant classes when ascospores were plated (overplating methods of NEWMEYER 1954) from crosses of the purple adenine mutants with a *hist-2—nic-2* double mutant strain. Experiments with 6 to 8 week-old crosses of mutant A2 gave evidence for equivalent recovery of complementary members of each of the three principal segregation classes, indicating comparable viability of the adenine-independent segregants under the plating conditions used.

Results of the analyses of these crosses are presented in Table 3. Since the number of colonies obtained on the individually supplemented plates represent the number of single crossovers plus any double crossovers, and the number of colonies on the doubly supplemented plates represent the total number of ascospores of all these genotypes, recombination frequencies for the individual intervals may be calculated directly (e.g., for B1 the percentage recombinatoin for the *hist-2—ad-3* interval $= 58/2703 \times 100 = 2.1$ percent).

Three mutants (B3, B7, B20) were omitted since too few ascospores for a plating analysis could be obtained from these crosses. Evidence from crosses of these mutants to wild type strain 73a suggests the presence of gross chromosome rearrangements in these strains as indicated by marked abortion patterns, low viability of ascospores, and marked deviation of the progeny from the expected ratio of 1:1. B21 was omitted since it grows extensively on minimal at 25° C.

Data from these experiments on the 19 crosses remaining indicate that one of the *ad-3A* mutants (A1) and 11 of the *ad-3B* mutants (B2, B5, B6, B8, B10, B11, B14, B15, B16, B17, and B18) have linkage relations similar to the mutant of known spontaneous origin, A2, and thus appear to have resulted from mutational changes restricted to either the *ad-3A* or *ad-3B* locus. With mutants A3, B1, and B13 crossing over in at least one interval was slightly or substantially reduced. These and unpublished data on the crosses of mutants B4 and B12 suggest the presence of chromosome rearrangements associated with each mutant, which result in an increase recovery of histidine or apparent niacin-requiring segregants,

TABLE 3

Recombination frequencies obtained for the hist-2—ad-3—nic-2 *regions from crosses of purple adenine mutants with a* hist-2 nic-2 *double mutant strain*

Cross:	a	hist-2	+	nic-2		
	A	+	ad-3	+		
	Total colonies per plating series				Recombination percentage	
Mutant number	M*	M + niacin	M + histidine	M + histidine + niacin	*hist-2—ad-3*	*ad-3—nic-2*
A1	0	44	59	2233	2.0±0.6†	2.6±0.6
A2	1	50	80	2810	1.8±0.4	2.8±0.6
A3	0	38	5	661	5.7±1.8	0.8±0.6
B1	0	58	4	2703	2.1±0.6	0.2±0.2
B2	1	52	67	2450	2.1±0.6	2.7±0.6
B4	11	170	21	517	32.9±4.1	4.1±1.8
B5	1	11	33	1015	1.1±0.6	3.3±1.2
B6	2	29	62	2322	1.2±0.4	2.7±0.6
B8	1	58	47	2162	2.7±0.8	2.2±0.6
B9	1	45	18	1221	3.7±1.0	1.5±0.6
B10	0	53	53	2215	2.4±0.6	2.4±0.6
B11	1	47	63	2143	2.2±0.4	2.9±0.8
B12	33	53	1385	2677	2.0±0.4	51.7±2.0
B13	0	27	0	1399	1.9±0.8	0.0±0.0
B14	3	31	61	1875	1.7±0.6	3.3±0.8
B15	0	2	8	274	0.7±1.0	2.9±2.0
B16	2	47	45	2365	1.9±0.6	1.8±0.6
B17	0	37	43	1829	2.0±0.6	2.4±0.8
B18	1	67	67	2497	2.7±0.6	2.7±0.6
B19	1	33	31	2428	1.4±0.4	1.3±0.4

* M = minimal.
† 95 percent confidence limits.

thus drastically altering the estimates of recombination in one of the regions adjacent to the *ad-3B* locus.

Comparative reversion experiments

Mutants resulting from gene mutation were expected to be capable of further change, either spontaneously or as a result of X-irradiation. Those mutations in the direction of wild type should grow on a minimal medium without the growth factor required by the parental strain. Thus, if any of the X-ray-induced or spontaneous mutants resulted from gene mutation, they should give rise to reversions under experimental conditions suitable for detection of these types.

The reversion experiments were performed on conidial isolates of each strain. (B21 was included by performing the experiment at 37° C.) The procedure was adapted from that used by GILES (1951). Centrifuged conidial suspensions of each mutant were irradiated with a dose of 25,000r (ca. 4000r/min, 6¼ min, 250 kv, 30 ma, 1 mm of aluminum filter) and then plated.

TABLE 4

Comparisons of spontaneous and X-ray-induced reversion in the individual purple adenine mutants (25° C)

			Control			X-irradiated with 25,000r		
Mutant number	Experiment number	Percentage survival	Viable conidia tested ($\times 10^6$)	Number of revisions	Reversions per 10^6 viable conidia	Surviving conidia tested ($\times 10^6$)	Number of reversions	Reversions per 10^6 surviving conidia
B1	Y90	69.0	160.3	0	0.0	127.8	0	0.0
A1	Y90	77.0	138.2	0	0.0	111.3	14	0.13
	Y121	76.5	193.5	0	0.0	148.2	13	0.09
B2	Y90	76.5	127.2	20	0.16	98.5	30	0.30
B3	Y90	91.8	123.0	0	0.0	126.7	0	0.0
B4	Y90	77.4	154.9	0	0.0	118.7	0	0.0
B5	Y90	89.0	209.4	0	0.0	184.5	0	0.0
B6	Y90	83.5	160.0	0	0.0	133.6	0	0.0
B7	Y90	64.0	194.9	0	0.0	124.2	0	0.0
B8	Y90	88.4	117.0	1	0.01	126.0	9	0.07
B9	Y121	74.5	191.0	0	0.0	142.8	0	0.0
B10	Y121	76.2	185.0	0	0.0	141.2	4	0.03
B11	Y121	86.5	226.0	0	0.0	196.0	17	0.09
B12	Y121	78.2	243.2	0	0.0	190.1	0	0.0
B13	Y121	80.0	245.0	0	0.0	194.0	0	0.0
B14	Y121	76.5	206.6	0	0.0	163.9	0	0.0
B15	Y121	74.0	215.0	0	0.0	159.1	0	0.0
B16	Y121	65.0	197.8	0	0.0	128.5	0	0.0
B17	Y121	72.5	212.0	0	0.0	149.9	63	0.42
B18	Y121	70.6	214.0	0	0.0	151.1	0	0.0
B19	Y121	83.4	166.5	0*	0.0	137.5	0*	0.0
A2	Y121	60.9	177.5	42	0.24	109.0	102	0.93
B20	Y121	80.4	166.0	0	0.0	134.1	0	0.0
A3	Y121	65.5	246.0	0	0.0	159.9	0	0.0
B21†	Y157	31.3	194.0	781	3.6	60.9	1485	22.2

* A few colonies resulting from contamination with *ad-3A* conidia have been eliminated from the tabulation.
† 35,000r.

Results of these experiments are presented in Table 4. In this series, nine of the mutants were mutable. A1 was used as a control in both sets of experiments and the agreement in survival and reversion frequencies indicates that all mutants were tested under comparable conditions.

Irradiated and control suspensions were plated at the original concentration and at a tenfold dilution to test for inhibition of prototrophs at these concentrations of conidia (GRIGG 1952). There was no evidence for any marked inhibition of this type at the conidial concentrations used.

The X-ray-induced reversion frequencies include the frequencies of spontaneous reversion. X-irradiation increases the frequency of reversion in mutants B2, B8, B21, and A2; but with A1, B10, B11, and B17, reversions were obtained only with X-irradiation. The reversion frequencies of mutant B21, however, are the highest of any mutants tested. The slightly higher dose (35,000r) accounts

for part of this increase, but the basically higher reversion frequencies of this strain undoubtedly are a reflection of a greater instability of this partial mutant.

Analysis of reversions

Biochemical and genetic studies on revertants produced in a number of inositol mutants (GILES *et al.* 1955) showed that these reversions fall into two categories: (1) partial or complete reverse mutations at the inositol locus, and (2) those caused by mutation at a suppressor locus.

Preliminary studies on purple adenine mutant 38701 (ultraviolet induced) (GILES *et al.*, *op. cit.*; KØLMARK and GILES 1955) showed that reversions at this locus (*ad-3A*) behave as alleles and are not caused by suppressor mutation. In general, reversions arising spontaneously or induced by ultraviolet, X-rays, or chemicals have resulted in the simultaneous restoration of adenine independence and loss of pigment formation. In addition, it has been shown that partial reversions can be obtained in which adenine independence is not restored completely. We wanted to know whether the same type of effects could be found for X-ray-induced reversions in X-ray-induced mutants and in the spontaneous mutants. Revertants of individual mutants were therefore tested to determine the level of adenine independence, and to find out whether reversion was caused by suppressor or reverse mutation.

Origin of homokaryotic strains

Since all the revertants were induced in macroconidial strains, they may be heterokaryotic when first isolated, as were those induced in the macroconidial strain of mutant 38701 (KØLMARK and GILES, *op. cit.*). Although adenine independence is dominant to adenine dependence in these heterokaryotic reversions, the heterokaryotic condition does not permit an accurate analysis for the level of adenine independence when the fluorescence and pigmentation tests are used.

Revertants from X-irradiated conidia of the mutable strains were selected initially, on the basis of qualitative biochemical tests, for obtaining strains ranging from those appearing almost completely adenine dependent to those appearing to be adenine independent. These revertants were crossed to wild type strain 73a or to a marked purple adenine strain, and serial or random ascospore isolations were made, respectively, to recover homokaryotic strains. Some of the homokaryotic revertants recovered and used in subsequent tests are: A1-R73, A1-R75, A1-R77, A1-R78, A2-R132, B2-R49, B10-R2, B10-R3, B10-R4, B11-R9, B11-R12, B11-R18, B17-R30, B17-R38 and B17-R63 (the first symbol is the mutant number, the second the isolation number of the revertant induced in that mutant).

Biochemical analyses on homokaryotic reversions

To determine the degree of adenine independence in X-ray-induced reversions, the homokaryotic strains were tested for adenine stimulation, fluorescence, and purple pigment formation at 25° and 33° C. These tests are the same as those used on the original purple adenine mutants and all the possibly heterokaryotic revertants recovered from the mutable strains.

The results of these tests have shown that X-ray-induced reversions were obtained in mutants A1, B10, B11, and B17, which appear to be completely adenine independent. The evidence also indicates that X-ray-induced reversion in two of the mutants (and possible four, since there is always some question about the origin of reversions recovered from X-irradiated conidia in mutants that revert spontaneously) resulted in partial adenine independence: A1-R77, A1-R78, B10-R2, B10-R3 (and B2-R49, and A2-R132, if they are of X-ray origin), all show varying degrees of adenine independence, most simply interpreted as resulting from varying levels of restoration of the normal adenine synthesis. These results are comparable to those of GILES *et al.* (1955) in which a partial restoration of inositol synthesis was demonstrated for an X-ray-induced revertant in mutant 89601 (induced by nitrogen mustard).

To determine whether the partial revertants were synthesizing enough adenine to permit growth at wild type rate, an experiment was performed on four revertants that showed varying degrees of partiality in the previous tests. The growth tube method (RYAN *et al.*, 1943) was used for comparisons of the linear growth rates of the revertants with that of wild type strain 74A and one of the parental purple adenine mutants, B10, at 33–34° C on minimal agar and minimal agar supplemented with adenine (Figure 1). All tubes were in duplicate and all experimental points represented averages of the measurements of total linear growth made over a period of four days.

Growth curves obtained on minimal agar for each of the revertants provide evidence for partial restoration of adenine synthesis in these mutants. The length of the lag period varies considerably from revertant to revertant, and the maximum growth rates achieved by each of the revertants in the period covered by the experiment are all less than that of wild type strain 74A. On minimal agar supplemented with adenine, all the revertants, in addition to B10, grow at wild type rate.

Genetic tests on reversions

Although the evidence presented by GILES *et al.* (1955) indicates that revertants induced by X-radiation in purple adenine mutant 38701 are not the result of suppressor mutation, additional evidence on the reversion of mutants, such as that on inositol mutants (GILES and PARTRIDGE 1953) or on tryptophan mutants at the *td* locus (YANOFSKY 1952), indicates that even within a series of alleles some of the mutants may revert by means of suppressor mechanisms and others may not. Since these same types of effects might apply to mutants in the *ad-3* region, revertants from each of the mutable strains were analyzed genetically to determine the nature of the reversion.

Crosses were made of each of the homokaryotic revertant strains with wild type strain 73a. Since all revertants except A2-R132 show no purple pigmentation at 25° C, the presence of the suppressor can be detected by incubating the isolates from these crosses at 25° C and scoring for the presence or absence of purple pigment. Accumulation of purple pigment in any of the isolates would indicate adenine dependence and would provide presumptive evidence for reversion

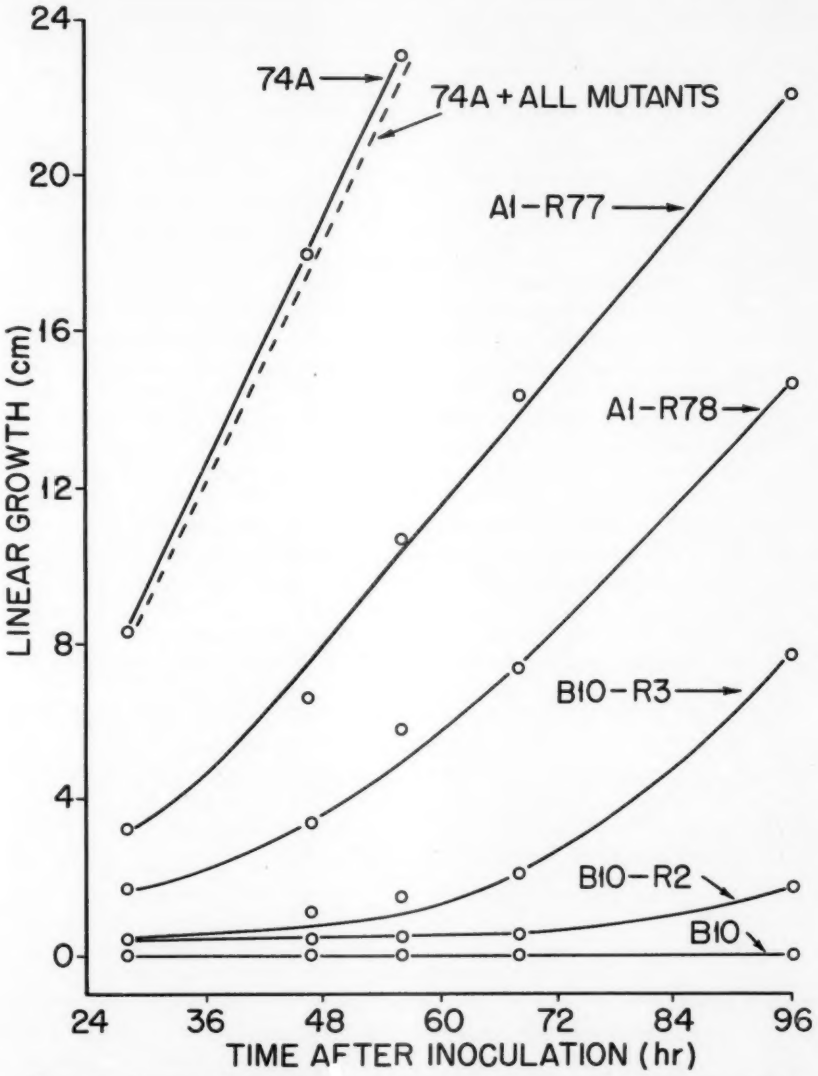

FIGURE 1.—Linear growth of homo aryotic partial revertants as compared with wild type strain 74A and purple adenine mutant B10 in growth tubes of minimal agar (solid lines) and minimal agar supplemented with 100 μg/ml of adenine (broken line) at 33–34° C.

caused by mutation at a suppressor locus. At least 200 random ascospore isolations were made from each of these crosses, and no evidence for the presence of suppressor mutation has been obtained, as indicated by the absence of purple pigment accumulation in the F_1 progeny.

Analysis of a partial reversion

The difficulty in obtaining more precise linkage relations for individual reversions was overcome to some extent by the recovery of a temperature-sensitive reversion from mutant B10, which exhibits a mutant phenotype more extreme in the 35–37° C range than indicated in Figure 1. Growth tube experiments on this revertant showed that heterokaryotic combinations of B10-R2 and A1 or A2 grow at less than wild type rate and show marked stimulation by the addition of exogenous adenine to the medium, whereas heterokaryons between the parental strain B10 and A1 or A2 show no exogenous adenine stimulation and grow at wild type rate (GILES 1956). Two alternatives seemed possible to explain the behavior of this revertant: (1) that mutational change confined to the *ad-3B* locus could result in interference with the complementary biochemical relation between the *ad-3A* and *ad-3B* loci in a heterokaryon, or (2) that the reverse-mutational event occurring in B10 was not confined to the *ad-3B* locus but modified the structure of the *ad-3A* locus as well.

Attempts were made to differentiate between these alternatives by crossing the revertant to a marked strain of A2, since, if B10-R2 was accompanied by mutation at the *ad-3A* locus, the frequency of adenine-independent recombinants recovered from such a cross would differ from the frequency recovered from a cross of the parental strain B10 with A2. In addition, crosses were made to a *hist-2 nic-2* double mutant to compare the linkage relations of the revertant with those of the parental strain in the *hist-2—ad-3* and *ad-3—nic-2* intervals.

The results of plating analyses, performed at 35–37° C, on these crosses are presented in Tables 5 and 6. Data from crosses of B10-R2 and B10 indicate no difference in the linkage relations of these two strains either with the *hist-2* or *nic-2* markers or with the *ad-3A* mutant A2. From the data available, the mutational changes that occurred in B10, resulting in the appearance of B10-R2, appear to have been confined solely to the *ad-3B* locus.

TABLE 5

Comparative linkage relations of a partial ad-3B *reversion and the parental strain in the* hist-2—nic-2 *region*

Mutant number	Total colonies per plating series				Recombination percentage	
	M*	M + niacin	M + histidine	M + histidine + niacin	*hist-2—ad-3*	*ad-3—nic-2*
B10-R2	2	167	357	6560	2.5±0.4†	3.9±0.4
B10	3	126	202	4952	2.5±0.4	4.1±0.6

* M = minimal.
† 95 percent confidence limits.

TABLE 6

Distribution of markers in adenine-independent isolates from crosses of a partial ad-3B *reversion and the parental strain with a marked strain of* ad-3A *mutant A2*

Cross:	a	hist-2	ad-3A	+	nic-2
	A	+	+	ad-3B	+

Mutant number	Total viable ascospores	Number of adenine-independent isolates	Recombination percentage	Genotypes of adenine-independent isolates			
				H+++	+++*N*	*H*++*N*	++++
B10-R2	23,180	22	0.19±.06*	0	21	0	1
B10	12,840	11	0.17±.08	0	10	1	0

* 95 percent confidence limits.

Mutability of a partial reversion

The extreme mutant phenotype of B10-R2 in the 35-37° C range permitted further tests for mutability on this X-ray-induced revertant. Since the genetic tests suggest that this revertant resulted from some form of intragenic alteration, it appeared desirable to determine whether this strain was mutable and could give rise to wild type revertants spontaneously or after X-irradiation. Reversion experiments were performed in the same manner as described previously, except that plates were incubated at 35–37° C, and conidia were irradiated with a dose of 35,000r. Reversion frequencies of 0.22 per 10^6 viable conidia and 0.86 per 10^6 surviving conidia were obtained for untreated and X-irradiated conidia, respectively. The partial revertant thus differs from the parental strain in that it is more unstable either spontaneously or after X-irradiation. No evidence is as yet available as to whether this strain mutates in the other direction, to adenine dependence.

DISCUSSION

The data from comparable forward-mutation experiments in which the filtration concentration technique was used to recover spontaneous and X-ray-induced purple adenine mutants demonstrated that substantially all the *ad-3B* mutants are of X-ray origin. Although the origin of any particular mutant could never be proved conclusively, the data do indicate that the incidence of purple adenine mutants of spontaneous origin at this locus must be extremely low and that X-irradiation has resulted in a substantial increase in the recovery of biochemical mutants of this type.

The origin of the *ad-3A* mutants recovered from X-irradiated conidia in these same experiments was not established with certainty since a number of *ad-3A* mutants recovered from untreated conidia. Two such mutants of uncertain origin, A1 and A3, were used in the present study.

Qualitative biochemical tests showed that purple adenine mutants at both loci have quite similar properties of pigment accumulation, fluorescence, and complete requirements for adenine or related compounds. Two of the mutants differ from

the others, however, in that they have only partial growth factor requirements. The recovery of this type of mutant indicates that, in the forward-mutation experiments, intermediate alleles were produced at the *ad-3B* locus in addition to those with complete requirements for adenine. The fact that two of the 21 *ad-3B* mutants were of this type is certainly no measure of the frequency of intermediate alleles at this locus, since the majority of mutant conidia of this type should germinate, grow, and be filtered off during the incubation period in minimal medium in the filtration-concentration experiments.

The linkage data on the individual mutant strains indicate the marked heterogeneity of genetic alteration obtained with X-irradiation. At least half the mutants appear to have resulted from such extragenic alterations as chromosomal rearrangements or deficiencies. Although the linkage data are not precise enough to indicate the presence of two separate loci in the *ad-3* region, since the *ad-3A* and *ad-3B* loci are probably less than 0.15 crossover units apart (DE SERRES 1956), in the current analysis, *ad-3A* and *ad-3B* mutants should have essentially the same linkage relations with the *hist-2* and *nic-2* markers. From the data available, 12 purple adenine mutants have linkage relations similar to the mutant of known spontaneous origin as shown in columns 2 and 3 in Table 7. These data are interpreted as indicating that these 12 mutants have resulted from intragenic alterations equivalent to point mutation.

Comparative reversion experiments with all the mutants showed that there is a marked correlation between irreversibility and abnormal linkage relations (Table 7). Of the 11 *ad-3B* mutants with apparently "normal" linkage relations, five are reversible either spontaneously or after X-irradiation, and six such mutants (B5, B6, B14, B15, B16, and B18) are stable. Present data are not adequate to determine whether any one of the latter group of mutants is mutable, but reverts at rates too low to have been detected in these experiments, or whether all the mutants are stable and thus may have resulted from such small deletions or rearrangements that no major alteration in linkage relations were observed with the markers used. In no case were reversions obtained in a mutant with linkage relations markedly different from mutant A2.

The genetic data for most of the reversions are not so precise as those for the parental strains, but the general genetic evidence available may be interpreted as indicating that the strains have resulted from alterations localized to the *ad-3* region, which at least in many instances may be considered intragenic. Data from more extensive analysis on a partial *ad-3B* revertant indicated that reversions do occur that are indistinguishable from the parental strain, on the basis of linkage relations with closely linked markers. The occurrence of such alterations on an intragenic level is substantiated by the subsequent mutability of such revertants either spontaneously or as a result of X-irradiation.

The results obtained in these experiments on the X-ray-induced reversibility of X-ray-induced mutants thus are in agreement with the findings of the early work in Drosophila and differ from the results of experiments reported by STADLER

TABLE 7

Correlation between linkage relations with hist-2 *and* nic-2 *markers and mutability of purple adenine mutants*

Mutant number	Recombination percentage		Reversions/10^6 survivors	
	hist-2—ad-3	*ad-3—nic-2*	Spontaneous	X-ray-induced
A1	2.0±0.6*	2.6±0.6	0.00	0.11
A2	1.8±0.4	2.8±0.6	0.24	0.93
B2	2.1±0.6	2.7±0.6	0.16	0.30
B5	1.1±0.6	3.3±1.2	0.00	0.00
B6	1.2±0.4	2.7±0.6	0.00	0.00
B8	2.7±0.8	2.2±0.6	0.01	0.07
B10	2.4±0.6	2.4±0.6	0.00	0.03
B11	2.2±0.6	2.9±0.6	0.00	0.09
B14	1.7±0.6	3.3±0.8	0.00	0.00
B15	0.7±1.0	2.9±2.0	0.00	0.00
B16	1.9±0.6	1.8±0.6	0.00	0.00
B17	2.0±0.6	2.4±0.6	0.00	0.42
B18	2.7±0.6	2.7±0.6	0.00	0.00
A3	5.7±1.8	0 8±0.6	0.00	0.00
B1	2.1±0.6	0.2±0.2	0.00	0.00
B3	....	....	0.00	0.00
B4	32.9±4.0	4.1±1.8	0.00	0.00
B7	....	....	0.00	0.00
B9	3.7±1.0	1.5±0.6	0.00	0.00
B12	2.0±0.4	51.7±2.0	0.00	0.00
B13	1.9±0.8	0.0±0.0	0.00	0.00
B19	1.4±0.4	1.3±0.4	0.00	0.00
B20	....	....	0.00	0.00

* 95 percent confidence limits.

(1941, 1944) and STADLER and ROMAN (1948) in maize, and by LEFEVRE (1950) in Drosophila.

There is evidence that the *a1* allele used for the experiments on the effect of X-irradiation on dominant mutation (STADLER 1944) may not have been so suitable for such a study as it was once thought. The *a1* allele is ordinarily quite stable and mutable only in the presence of the *Dt* gene (RHOADES 1941), or by the breakage-fusion-bridge cycle (MCCLINTOCK 1951) of chromosome 9. MCCLINTOCK suggests that reversions of *a* and *A* are ascribable to changes in an inhibited state of *a* controlled by a type of dissociator-activator system. Evidence presented by STADLER (1951) indicates that the *A′* alleles that arise from the *a* allele under the influence of *Dt* are qualitatively different from normal *A* alleles, since the *A′* alleles are mutable in the presence of *Dt* whereas normal *A* alleles are not. The evidence from all these studies suggests that mutation of the *a1* allele may occur *only* under the influence of *Dt* or "Dt-like" factors. If this is the case, then *a1*

allele does not meet the requirements discussed by STADLER (1941, 1944), since critical evidence of failure to mutate to a dominant allele can only be obtained from recessive alleles capable of spontaneous mutation. In addition, alternative explanations are possible for the results of the experiments on X-ray-induced mutation of the *A1* allele (STADLER and ROMAN 1948). In choosing *A* losses from a total of about 415 anthocyanin-free seedlings for cytogenetic study, selection was made for those F_1 plants with nondefective pollen. A cytogenetic analysis on two of these plants, and one with segregating subnormal pollen, demonstrated that these *A* losses were caused by deficiencies and not gene mutation of the *A1* allele. However, the possibility also exists, as was pointed out by these authors, that there were instances of gene mutation of *A1* among the population of *A* losses observed, but because of coincident but independent chromosomal alterations, they were not haploviable, or else gave such reduced gametophytic transmission that they were eliminated from the cytogenetic analysis.

In the experiments of LEFEVRE (1950), the requirements established by STADLER (1941, 1944) for obtaining critical evidence on the effects of X-rays on dominant and recessive mutation have not been fulfilled. No evidence is presented demonstrating that the recessive alleles used for study of X-ray effects on various loci in the X chromosomes are capable of mutation spontaneously. Although attempts to induce reversions in white-eye mutants of three different origins were unsuccessful, in none of these three stocks was a spontaneous reversion observed.

Although the present studies showed that a large number of X-ray-induced *ad-3B* mutants are stable, they have also shown that a substantial portion (25 percent in the present sample) are capable of either spontaneous or X-ray-induced reversion. Furthermore, the evidence suggests that the X-ray-induced alterations that have been observed are caused by gene mutation, or to localized alterations that simulate gene mutation.

The genetic nature of X-ray-induced changes that simulate gene mutation has been the subject of much discussion. In a review of the gene concept, and the role of radiation studies in forming that concept, STADLER (1954) concludes that "we have no positive criterion to identify mutations caused by a change within the gene and that the alterations interpreted as gene mutations in experiments are merely the unclassified residue that cannot be proved to be due to other causes."

The work of MCCLINTOCK (1951, 1955) shows quite clearly that, in maize, instability at a locus may be attributable to a number of factors. Among those cases that she has studied, there are loci at which the instability is under autonomous control, and others at which the instability is under the control of mutators or suppressors such as *Ac* or *Spm*. There appear to be no adequate criteria for distinguishing between those loci at which mutation is caused by alteration of the genetic material within a locus and those at which mutation is caused by alterations that do not directly alter the structure of the genes themselves but that actually affect the functioning of controlling elements located at or near such genes.

Further evidence presented by MCCLINTOCK (1951) would suggest specific

control of certain types of genic action by heterochromatin. Transposed to new locations by the breakage-fusion-bridge cycle, these heterochromatic elements continue their specific control, but affect the action of genic components at the new locations. Radiation-induced position effects in Drosophila (see LEWIS 1950, for a general review) resulting from transposition of heterochromatin, give evidence that some of the same general types of effects, obtained with the breakage-fusion-bridge cycle, can be induced by X-irradiation.

Apparently, then, even in cases where the absence of any detectable disturbance in linkage relations and failure to obtain evidence for suppressors would suggest that reverse mutation in a particular mutant resulted from an "intragenic alteration," other interpretations are possible. Such results could be obtained either by mutation of a very closely linked "suppressor" which restored the synthesis interrupted by the original mutation, or by the removal of transposed heterochromatin, which could have produced the original "mutant" phenotype and by its very removal restores the normal phenotype. We have no simple or generally useful criteria for distinguishing between these alternatives.

In light of these observations, it is perhaps not surprising that STADLER (1954) commented that "in the study of gene mutation, we are for the present in an anomalous position. A mutant may meet every test of gene mutation, and yet, if it is not capable of reverse mutation there is ground for the suspicion that it may be due to gene loss, while, if it is capable of reverse mutation, there is ground for the suspicion that it may be due to an expression effect. The only escape from this dilemma is through a more intensive study of the mutations of specific genes selected as best suited to detailed genetic analysis, in hope of developing more sensitive criteria for the identification of gene mutation."

SUMMARY

(1) A series of 24 purple adenine mutants obtained in forward-mutation experiments by the "filtration-concentration" technique were used to investigate the reversibility of X-ray-induced mutants in the *ad-3* region in *Neurospora crassa*. Three are *ad-3A* mutants and 21 are *ad-3B* mutants.

(2) Comparable experiments with untreated and X-irradiated conidia showed that substantially all *ad-3B* mutants recovered from filtration of X-irradiated conidia are of X-ray origin, whereas the origin of *ad-3A* mutants recovered in the same experiments is questionable.

(3) Two of the X-ray-induced *ad-3B* mutants have partial requirements for adenine; B21 grows extensively, and B16 shows limited growth on minimal medium at 25° C, whereas the remaining mutants show complete requirements for adenine.

(4) Linkage data from crosses of each of the *ad-3B* mutants have shown that 11 of the *ad-3B* mutants have "normal" linkage relations in the *hist-2—ad-3* and *ad-3—nic-2* intervals, and thus appear to have resulted from intragenic alteration; however, since recombination in one or both of these intervals was altered in crosses of the remaining mutants these appear to have resulted from some form of extragenic alteration.

(5) Comparative reversion experiments showed that 5 of the 11 *ad-3B* mutants with "normal" linkage relations are mutable and revert back to wild type either spontaneously or after X-irradiation, whereas all the mutants with "abnormal" linkage relations are stable.

(6) Biochemical analysis of X-ray-induced *ad-3B* mutants showed that partial revertants were obtained in addition to those appearing to be complete revertants to wild type.

(7) Genetic analyses of X-ray-induced *ad-3B* revertants showed that partial and complete reversion to wild type results, in many instances, from localized changes in the *ad-3B* region, indicating that X-rays are capable of inducing changes most simply interpreted as reverse (gene) mutations. No evidence was obtained indicating that reversion occurs through suppressor mutation.

(8) Reversion tests on an X-ray-induced partial *ad-3B* revertant showed that this strain is mutable and gives rise to wild type reversions spontaneously and at higher rates after X-irradiation.

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THE EFFECT OF AGE ON THE RELATIONSHIP BETWEEN COINCIDENCE AND CROSSING OVER IN DROSOPHILA MELANOGASTER

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BRIDGES (1915), working on a two-strand model, noticed that recombination in general decreases as the age of the female increases to about 16 days and then increases again following a wave-like pattern. Further, he produced evidence suggesting that interference does not always bear the relationship to recombination which one would expect and suggested a model according to which crossing over was brought about by twisting of the chromosomes round one another. The twist would produce nodes at which the chromosomes would eventually be broken and a rejoin of A to B would result in a crossover. The distance between twists he called internode length; the chance that crossing over takes place at a node which is not necessarily unity, he called the coefficient of crossing over. He points out that a study of interference and crossing over will show whether changes in the frequency of crossing over are due to changes in internode length or the coefficient of crossing over. If the former is responsible, there will be a change in intereference; if the latter, there will be none. In his first paper his data were not adequate to show definitely what changes in coincidence, the measure of interference used, had in fact taken place. In a later paper (1927) working with the third chromosome he unfortunately chose a length which straddles the centromere and once more his data are not conclusive. If it can be established that changes in recombination and coincidence can be independent of one another, it will support BRIDGES' suggestion that there are nodes of crossing over on any particular pair of chromosomes, not necessarily localized at the same point in all pairs, at which crossing over may take place but does not necessarily do so.

In a previous paper (RENDEL 1957) a comparison of recombination and coincidence on the X chromosome with and without the *Cy* inversion was made. It was shown that on the probit scale the relationship between coincidence and recombination follows a straight line as standard map length is increased. That is, as more nodes are included in the region marked, coincidence increases in a regular way. This relationship takes account of the fact that as distance increases doubles will be missed. When an environmental agent, such as age or an inversion in another chromosome, is used to increase recombination in a particular section, if an increase in the number of nodes present is responsible, the same relationship between recombination and coincidence should hold. If it does not hold, a two stage process of some kind is indicated as suggested by BRIDGES. When the *Cy* inversion

was used to increase recombination on the X, internode length decreased, i.e. number of nodes increased but not to the extent expected. That is to say, coincidence did not rise to the point indicated by the graph. Some increase in the coefficient of crossing over must be assumed. In this paper the effect of age has been examined. BRIDGES found this to be zero for the X chromosome in his stocks but the frequency of crossing over in later hatched flies in the crosses with the *Cy* inversion suggested that the effect of age might be worth re-examining in the stocks used here.

METHODS AND RESULTS

F_1 cultures in which the female progeny were of the constitution

$$\frac{sc\ ct\ v,}{+\ +\ +}\quad \frac{sc\ +\ +,}{+\ ct\ v}\quad \frac{+\ ct\ +}{sc\ +\ v}\quad \text{and}\quad \frac{+\ +\ v}{sc\ ct\ +}$$

were set up in halfpint milk bottles containing standard medium. These cultures were segregating for the *Cy* inversion, and as they emerged, normal and *Cy* sisters from the same culture were set up one female to a bottle. These females were mated to *sc ct v* males and transferred every four days to a fresh bottle for four transfers. Any female not having progeny in all four bottles was discarded. The F_2 progeny were scored and the results are shown in the tables.

The F_1 females were made up so that the recessive genes at the three heterozygous loci were in coupling and repulsion in all possible combinations. This was done to discount the effect of differential viability between the crossover and non-crossover classes, which, being of different genotype, will have different mortalities. It is possible that having the recessive genes differently distributed between the two X chromosomes of the heterozygous female may alter the crossover

TABLE 1a

Crossing over in X chromosome in females normal for second chromosome

F_1 Female	Recombination classes ++				Proportions				
	I	II	I & II	TOTAL	I	II	I & II		
$\frac{sc\ ct\ +}{+\ +\ v}$	85	95	3	739	.1150	.1286	.0041		
$\frac{sc\ +\ v}{+\ ct\ +}$	115	64	2	695	.1655	.0921	.0029	First laying period	
$\frac{sc\ ct\ v}{+\ +\ +}$	266	172	9	1156	.2301	.1488	.0078		
$\frac{sc\ +\ +}{+\ ct\ v}$	63	64	1	429	.1469	.1492	.0023		
					.1644	.1297	.0043	Recom 1	16.87%
								Recom 2	13.40%
								Coincidence	0.1902

TABLE 1a—Continued

$\frac{sc\ ct\ +}{+\ +\ v}$	80	79	4	709	.1128	.1114	.0056		
$\frac{sc\ +\ v}{+\ ct\ +}$	132	90	4	973	.1357	.0925	.0041	Second laying period	
$\frac{sc\ ct\ v}{+\ +\ +}$	237	164	19	1376	.1722	.1192	.0138		
$\frac{sc\ +\ +}{+\ ct\ v}$	109	70	8	594	.1835	.1178	.0135		
					.1511	.1102	.0093	Recom 1 Recom 2 Coincidence	16.04% 11.95% 0.4852
$\frac{sc\ ct\ +}{+\ +\ v}$	38	57	2	543	.0700	.1050	.0036		
$\frac{sc\ +\ v}{+\ ct\ +}$	72	60	9	687	.1048	.0873	.0131	Third laying period	
$\frac{sc\ ct\ v}{+\ +\ +}$	163	165	9	1412	.1154	.1169	.0064		
$\frac{sc\ +\ +}{+\ ct\ v}$	89	80	6	544	.1636	.1471	.0110		
					.1135	.1141	.0085	Recom 1 Recom 2 Coincidence	12.20% 12.26% 0.5683
$\frac{sc\ ct\ +}{+\ +\ v}$	17	45	0	390	.0436	.1154	....		
$\frac{sc\ +\ v}{+\ ct\ +}$	67	59	7	929	.0721	.0636	.0075	Fourth laying period	
$\frac{sc\ ct\ v}{+\ +\ +}$	67	54	4	581	.1153	.0929	.0069		
$\frac{sc\ +\ +}{+\ ct\ v}$	85	63	4	454	.1872	.1388	.0088		
					.1046	.1027	.0058	Recom 1 Recom 2 Coincidence	11.04% 10.85% 0.4842

TABLE 1b

Crossing over in X chromosome in females having the second chromosome Cy *inversion*

Female	Recombination classes I	II	I & II	TOTAL	*Cy* Proportions I	II	I & II		
$\frac{sc\ ct\ +}{+\ +\ v}$	171	112	10	732	.2336	.1530	.0137		
$\frac{sc\ +\ v}{+\ ct\ +}$	91	67	7	409	.2225	.1638	.0171	First laying period	
$\frac{sc\ ct\ v}{+\ +\ +}$	308	210	18	1195	.2577	.1757	.0151		
$\frac{sc\ v\ +}{+\ +\ ct}$	124	89	4	685	.1810	.1299	.0058		
					.2237	.1556	.0129	Recom 1 Recom 2 Coincidence	23.66% 16.85% 0.3236
$\frac{sc\ ct\ +}{+\ +\ v}$	200	134	14	941	.2125	.1424	.0149		
$\frac{sc\ +\ v}{+\ ct\ +}$	111	77	7	538	.2063	.1431	.0130	Second laying period	
$\frac{sc\ ct\ v}{+\ +\ +}$	325	229	24	1331	.2442	.1721	.0180		
$\frac{sc\ v\ +}{+\ +\ ct}$	123	85	3	680	.1809	.1250	.0044		
					.2110	.1457	.0126	Recom 1 Recom 2 Coincidence	21.36% 15.83% 0.3680
$\frac{sc\ ct\ +}{+\ +\ v}$	132	115	5	908	.1454	.1267	.0055		
$\frac{sc\ +\ v}{+\ ct\ +}$	98	56	8	464	.2112	.1207	.0172	Third laying period	
$\frac{sc\ ct\ v}{+\ +\ +}$	283	211	22	1433	.1975	.1472	.0154		
$\frac{sc\ v\ +}{+\ +\ ct}$	95	64	6	513	.1852	.1248	.0117		
					.1844	.1299	.0125	Recom 1 Recom 2 Coincidence	19.69% 14.24% 0.4458

TABLE 1b—Continued

Crossing over in X chromosome in females having the second chromosome Cy *inversion*

	Cy								
	Recombination classes				Proportions				
Female	I	II	I & II	TOTAL	I	II	I & II		
sc ct + / + + *v*	103	100	4	782	.1317	.1279	.0051		
sc + *v* / + *ct* +	86	75	3	668	.1287	.1123	.0045	Fourth laying period	
sc ct v / + + +	230	131	13	946	.2431	.1385	.0137		
sc v + / + + *ct*	105	74	2	567	.1852	.1305	.0035		
					.1722	.1278	.0067	Recom 1	17.89%
								Recom 2	13.40%
								Coincidence	0.2794

frequency, quite apart from effects of genotype on viability. If this is true it will not be possible to distinguish such an effect from differential viability. The best way of allowing for both effects seems, therefore, to calculate the fraction of the population belonging to each crossover class separately for all four types of crosses and then to take an unweighted average of these fractions. By this method undue weight may be given to the cross which has the smallest number of flies but any error should be unbiased.

The tables show that the highest rate of crossing over takes place in the eggs laid during the first four days. Crossing over declines steadily from then on till the 12th–16th day, as found by BRIDGES (1927). Coincidence on the other hand is least in the eggs laid early and increases to the twelfth day after which it drops again. The same trends are followed in the progeny of both *Cy*/+ and +/+ but the effect of age on coincidence is more noticeable in the +/+ cultures. Although the crossing over frequency in the *Cy*/+ cultures is higher than in the +/+ the coincidence is much lower except during the first laying period. Thus age reduces crossover frequency and increases coincidence whereas the *Cy* inversion does the reverse. It may be noted that whereas in the *Cy* cultures the actual proportion of double crossovers is very similar in all but the last laying period, the proportion of double crossovers in the +/+ cultures is very much higher in the second and third laying period. An analysis of variance and covariance based on the percent recombination and the coincidence values shows that the covariance term is always strongly negative. In the *Cy* cultures the effect of age and cross on coincidence and recombination are highly significant. In the +/+ cultures neither the effect of age or cross on coincidence is significant, but the covariance term is always strongly negative, and the effect of age and cross on recombination highly significant.

DISCUSSION

Before discussing the bearing of these findings on theories of crossing over at meiosis the possibility that observed changes in coincidence are due to crossing over at mitosis must be considered. WHITTINGHILL (1955) has shown that gonial crossing over during mitosis could theoretically have an effect on coincidence. He has, however, shown that the presence of an inversion did not affect the frequency of somatic crossing over brought about by radiation (WHITTINGHILL 1947) so that it is unlikely that the effects of the *Cy* inversion reported here were brought about during gonial mitosis. SCHWARTZ (1954) found that twin spotting resulting from somatic crossing over in attached-X individuals increased with age and put this down to increased sister strand crossing over, which would not be detectable in the experiment reported here. BROWN and HANNAH (1952) also suggested that age increases sister strand crossing over. They found aged females mated to males carrying a ring X chromosome had progeny with a greater number of mosaics than young females. These mosaics almost all appeared to owe their origin to the loss of the ring chromosome. The elimination of the ring was put down to sister strand crossing over as the authors did not believe it could be due to crossing over between the ring and its normal partner, since there is little if any pairing between them at the time the loss occurred, and further because such an interchange should give rise to more than one type of mosaic and only one was found.

Thus, although these authors have demonstrated that age affects events taking place at mitosis they do not believe that it increases the over-all frequency of mitotic crossing over. But let us suppose for the sake of argument that it does, and that the tenfold increase with age in twin spotting found by SCHWARTZ in $\overline{XX}$ females was due to an increase in frequency of mitotic crossing over. Then if the number of crossovers in male Drosophila can be used as a guide to the frequency of gonial crossing over in females we may expect 1 in 10,000 offspring to be the result of gonial crossing over. As in the experiment reported here where only half the X chromosome is marked, this might show up in 1 in 20,000 flies. If age increases the rate tenfold the frequency becomes 1 in 2,000. Of these, only 15 to 20 percent will appear as double crossovers, depending on the rate of meiotic interchange, and so only very few extra doubles, the result of somatic crossing over, are expected throughout the whole experiment. This would not noticeably alter the results and we may safely assume that any effects observed are due to events happening at meiosis.

This being so, we have to explain how coincidence can become greater as crossover frequency becomes less with age, and how the reverse happens when inversions are present in another chromosome. The effect of age on recombination was quite large. In crosses without *Cy*, recombination dropped from 30 to 22 percent, and in the stocks with *Cy*, from 41 to 31 percent. This might be expected to reduce coincidence from about .24 to about .07 and from about .5 to about .24 respectively. In fact it rose from .2 to .5 in the cross without *Cy* and from .32 to .44 before falling to .27 in the crosses with *Cy*. It has been suggested that such events

would be possible if crossing over were determined in two stages. First, points down the length of each bivalent are determined at which an interchange may take place but at which it does not necessarily do so, and second, the frequency with which interchange takes place at these points may be higher or lower according to circumstances. It would then be possible to vary coincidence and crossing over frequency to some extent independently. Coincidence would depend on the average distance between potential points of crossing over, and crossing over frequency would, in addition, be affected by the frequency of interchange at each such point.

Three main mechanisms have been suggested as responsible for crossing over. BELLING (1933) suggested that interchange took place between new strands during reduplication as a result of new genes becoming joined after formation to the gene string of the opposite chromosome. BRIDGES (1915) and DARLINGTON (1935) have suggested that torsion due to coiling will break the chromatids, the broken ends of which will then join up with the chromatid of the other chromosome. HALDANE (1954) has suggested that during reduplication at meiosis the gene string may become discontinuous, being held in place by a skeletal structure, possibly a template formed on the original gene string, which remains continuous. Four discontinuous gene strings are then formed. These will then join into four continuous threads and the way they do so will determine the amount of crossing over; eventually the skeletal structure becomes discontinuous and the gene threads can separate.

Clearly, on BELLING's and HALDANE's hypotheses, the position of one strand relative to a homologue will make a great difference to the chance of a join being formed which results in a crossover and it could well be that the degree of internal and relational coiling of the chromatids will determine the number of points where homologous genes on different chromatids are close enough to permit them to join up with the neighbouring genes of their partner chromatid. This would be the distance between nodes of BRIDGES and, as on his model, dependent on relational coiling. If this picture is basically correct, it should be possible to relate the distance between nodes of relational coils with the minimum distance between two crossovers. Thus the least distance between double crossovers on the X chromosome of *D. melanogaster* appears to be about ten units, and the coiling seen in the Xs in salivary chromosomes appears from published pictures to have between six and seven nodes along the chromosome which is thus divided into units between the nodes of about the right length. However, as the relationship between coiling in salivary chromosomes and coiling at meiosis is not known this observation cannot be taken very seriously.

BRIDGES' idea that the twisting of the chromosomes actually broke them assumes that two breaks will always occur at exactly the same place. BELLING's theory only fits four strand crossing over if sister strand crossing over also takes place.

Evidence that sister strand crossing over does take place is not conclusive, though recently (e.g. SCHWARTZ *loc. cit.*) some very suggestive arguments have

been put forward in its favour. These, however, do not rule out the possibility that even if sister strand crossing over does take place, four strand crossing over may take place directly. There seems no reason to suppose that a "new" chromatid can join with an "old" one if it is a sister strand but not if it is a homologous non-sister strand unless it can be conclusively proved to be so.

On the whole HALDANE's theory, which is in essence the same as BELLING's but permitting four strand crossing over, seems to fit all the factors so far brought forward better than any other.

SUMMARY

The effect of age on crossing over is the reverse of its effect on coincidence.

Where age is taken into account the effect of the *Cy* inversion on crossing over is also the reverse of its effect on coincidence.

The bearing of this on the theories of crossing over is discussed.

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A NOTE ON THE FREQUENCY DISTRIBUTION OF TETRADS BY RANK IN DROSOPHILA MELANOGASTER

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TETRAD frequencies for free X chromosomes, reasonably well marked for their entire genetic length, have been computed by WEINSTEIN (1936). A typical set of values are: no-exchange tetrads (E_0) = 0.056, one-exchange tetrads (E_1) = 0.485, two-exchange tetrads (E_2) = 0.429, and tetrads of rank greater than two (E_3) = 0.030. In tetrad frequencies obtained from structurally abnormal X chromosomes, e.g., heterozygous inversions, exchange is reduced. This is indicated, generally, by an increased frequency of zero (and, depending on the amount of the reduction, perhaps single) exchange tetrads at the expense of the multiple exchange classes. There are, however, two structurally aberrant chromosomes, the exchange values from which do not accord with any others known; namely, the reversed acrocentric compound X chromosome and the reversed compound ring X chromosome. The former is structurally similar to an attached-X chromosome with the centromere subterminal instead of medial, and the latter is also similar to an attached-X chromosome, but with the free chromosome ends connected by a heterochromatic segment. The two chromosomes, then, are structurally very much alike both in the way they can synapse and in that they both contain a heterochromatic segment between the two component chromosomes of the compound. Both chromosomes give a frequency distribution of tetrads characterized by a high frequency of tetrads of ranks zero and two (or perhaps greater than two) with a low frequency of single exchange tetrads (SANDLER 1954, 1957). Such a distribution is manifestly very strange.

Now, of course, the first possibility that must be considered is that this distribution is only apparent; that is, some mistaken assumption has been used in analyzing the data and arriving at the exchange values. The possibility would exist, for example, that single exchange products from these chromosomes are not recoverable, or that chromatid interference operates in these compounds. Although it is certainly not possible to eliminate all such possibilities, the reports on those chromosomes show that they are rather unlikely, and that the most reasonable supposition at the present time is that the strange distribution inferred from the data is approximately the true one. Existing data are unfortunately not precise enough to distinguish between a low frequency of single exchanges and an absence of singles.

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If single exchanges are completely absent, it would seem that the explanation required is a physical (as opposed to a stochastic) model according to which it would physically be extremely difficult, or impossible, for a single exchange to occur in these compounds. If, on the other hand, there is really some low frequency of singles (as indeed the data would appear to indicate), then it would seem that a model is required that renders singles, not impossible, but only relatively improbable. In this note, we propose to explore one such model. It should be emphasized that this is not meant to imply that the physical model will not be required—we merely wish to consider the second alternative.

THE PROPOSED MODEL

Consider first the case of normal, free, X chromosomes. Imagine that the physical basis of crossing over (chromosome replication, for instance) starts at one end of the chromosome arm (which end is not, for the moment, important) and proceeds toward the other end. We suppose that in every genetic unit of length, there is some constant probability of an exchange, α. After an exchange has occurred, chromosome replication continues in the same direction, but the per-unit probability of an exchange is now different, say β. After a second exchange, the probability reverts to α, after a third exchange, to β again, and so forth. Now, if it is possible to consider the frequency distribution of tetrads from normal X chromosomes as being generated by these two probabilities, it seems likely that $\alpha > \beta$. If this is so, then the distribution from the reversed acrocentric and reversed ring compounds might be generated by this same model but with the two probabilities (α and β) interchanged. This might mean, for example, that the interstitial heterochromatin of the compound is synaptically equivalent to the first exchange. The required distribution could come about because the probability of the first exchange is low (β), giving rise to an appreciable number of no-exchange tetrads, but, given an exchange, the probability of a second exchange is high (α), which could mean that most of the single-exchange tetrads would be converted to the double-exchange class.

It is now required that we express the probabilities of the different rank tetrads (for normal chromosomes) in terms of the two parameters, α and β. We must, however, first consider the matter of interference. Ideally, we should like to incorporate into the probability expressions a function of the distance from any exchange, such that the probability of any exchange (except the first) at any point would be given by the appropriate parameter (β, if, for example, the exchange being considered is the second one) multiplied by the function evaluated at the point being considered. This approach was not used because the incorporation of such functions (even relatively simple ones) lead to complex products which are, in general, intractable. Instead, the true function, whatever it may be, has been approximated by a step function whose value is zero for i units after every exchange and one thereafter. This can be handled reasonably easily.

We now write down the probabilities of exchanges of ranks 0, 1, 2, and more

than 2—P_0, P_1, P_2, and P_3. It follows directly from the model that, for a chromosome N units long, $P_0 = (1 - a)^N$.

To obtain P_1, we consider separately all the possible cases; that is, that the one required exchange in the tetrad occurs in each of the N genetic units. Thus, for the first $(N - i)$ units we have,

Unit in which the exchange occurs	*Probability*
1	$(1-a)^0\, a\, (1-\beta)^{N-i-1}$
2	$(1-a)^1\, a\, (1-\beta)^{N-i-2}$
3	$(1-a)^2\, a\, (1-\beta)^{N-i-3}$
.	.
.	.
.	.
k	$(1-a)^{k-1}\, a\, (1-\beta)^{N-i-k}$
.	.
.	.
.	.
$N-i$	$\dfrac{(1-a)^{N-i-1}\, a\, (1-\beta)^0}{a \sum_{k=1}^{N-i} (1-a)^{k-1}\, (1-\beta)^{N-i-k}}$

and for the remaining i units, in which the probability of a second exchange is zero by the definition of i, we have,

Unit in which the exchange occurs	*Probability*
$N-i+1$	$(1-a)^{N-i}a$
$N-i+2$	$(1-a)^{N-i+1}a$
.	.
.	.
.	.
r	$(1-a)^{N-i+r}a$
.	.
.	.
.	.
N	$\dfrac{(1-a)^{N-1}a}{a \sum_{r=0}^{i-1} (1-a)^{N-i+r}}$

In total then,

$$P_1 = a \sum_{k=1}^{N-i} (1-a)^{k-1}\, (1-\beta)^{N-i-k} + a \sum_{r=0}^{i-1} (1-a)^{N-i+r} .$$

By the same type of argument,

$$P_2 = \alpha \sum_{s=1}^{N-2i-1} (1-\alpha)^{s-1} \left[\beta \sum_{k=1}^{N-2i-s} (1-\beta)^{k-1} (1-\alpha)^{N-2i-k-s} + \beta \sum_{r=0}^{i-1} (1-\beta)^{N-2i-s+r} \right]$$

$$+ \alpha \beta \sum_{t=0}^{i-1} (1-\alpha)^{N-2i-1+t} \sum_{x=0}^{i-t-1} (1-\beta)^{x} ,$$

and, finally,

$$P_3 = 1 - P_0 - P_1 - P_2.$$

Evaluating the indicated summations, we find that

$$P_0 = (1-\alpha)^N$$

$$P_1 = \frac{\alpha(1-\beta)^{N-i} - \beta(1-\alpha)^{N-i}}{\alpha-\beta} - P_0$$

$$P_2 = (1-\alpha)^{N-2i-1} \left[1 + \frac{(N-2i-1)\,\alpha\,\beta}{\beta-\alpha} \right] + \frac{\alpha^2\,(1-\beta)}{(\beta-\alpha)^2}$$

$$\left[(1-\beta)^{N-2i-1} - (1-\alpha)^{N-2i-1} \right] - (P_0 + P_1)$$

$$P_3 = 1 - P_0 - P_1 - P_2.$$

According to the proposed model, the frequency distribution of tetrads from the reversed acrocentric and reversed ring compounds is generated by these same equations with α replaced by β, and β replaced by α.

Some numerical estimates of α and β

Typical tetrad frequencies for free X chromosomes are given in Table 1, and for reversed acrocentric chromosomes in Table 2. Reversed rings behave very much like reversed acrocentrics, but certain complications (see SANDLER 1957) do not allow a direct computation of exchange values.

TABLE 1

The frequency distribution of tetrads by rank for free X chromosome tetrads heterozygous for sc ec cv ct v g f

	Experiment A		Experiment B	
Rank of tetrad	Number	Relative frequency	Number	Relative frequency
0	1,709	0.06	1,522	0.09
1	17,982	0.64	10,038	0.62
2	8,076	0.28	4,464	0.28
3	472	0.02	112	0.01

In experiment B, the chromosomes were actually heterozygous for *s* instead of *g*, and in addition *car* and *bb*. The experiment has been corrected to include only the *sc* to *f* region so that it is comparable with experiment A (data from WEINSTEIN 1936).

TABLE 2

The frequency distribution of tetrads by rank for reversed acrocentric compound X chromosomes

Rank of tetrad	Experiment A		Experiment B		Experiments A + B	
	Number	Relative frequency	Number	Relative frequency	Number	Relative frequency
0	1,059	0.39	1,399	0.48	2,458	0.44
1	454	0.17	222	0.08	676	0.12
2+	1,200	0.44	1,304	0.44	2,504	0.44

Experiments A and B are two different reversed acrocentric lines, both of which are marked in the same way, and both of which carry a homolog for the compound. (Data from SANDLER 1954).

With respect to numerical values of α and β, a few points must be considered. First of all, the exchange values given in both Tables 1 and 2 must have rather high errors attached to them because the method of tetrad analysis (especially for reversed acrocentrics) involves the propagation of statistical error. Moreover, since the equations given here are not algebraically solvable, any iterative solutions obtained involve a further propagation of this error. Furthermore, experimentation with these equations shows that they are rather sensitive to changes in α and β even in the third decimal place. For example, the value of α for the case of free X chromosomes comes directly from the equation for P_0. In experiment A of Table 1, $\alpha = 0.047$; for experiment B, $\alpha = 0.041$. Although the results from these two experiments are significantly different, the experiments are genetically the same and are certainly as comparable as either is with the reversed acrocentric experiments. Thus it would seem that, whereas the iterative solutions to the equations require a high degree of accuracy of α and β, this accuracy is, for the most part, biologically meaningless. A third factor is that only integral values of i have been used in these solutions, which must add to the imprecision in the estimates of α and β. Finally, it should be noted, that whereas the value of N (taken here to be the genetic length of the chromosome) is known fairly well for free X chromosomes ($N = 57$ for the region from *sc* to *f*), only a very rough guess can be made for the case of the reversed acrocentric. The value chosen here was 67 units. It is clear that estimates of i will be affected by the lack of precision in N. It should be noted that although the estimates of α and β themselves do, of course, depend on N, the ratio, β:α is, for small α and β (in the range of interest here) only very slightly dependent on N, and it is this ratio that is of primary theoretical interest.

For these reasons it seems that the most that can be expected is a general agreement in the values of α, β, and i as computed from the different sets of data. The results of such computations are given in Tables 3 and 4, which show that, for α between 0.04 and 0.05 and for β between 0.01 and 0.02, all the data are approximately satisfied.

The value of i obtained for all cases is reasonable. The difference between the value for reversed acrocentrics (11) and normal chromosomes (17) may very well not be real. If it is real, it could be that interference is simply different in

TABLE 3

Numerical estimates of α, β and i from data from free X chromosomes

Experiment	Rank	Observed frequency	Calculated* frequency	Estimates of parameters
A	0	0.06	0.06	$\alpha = 0.047$
	1	0.64	0.64	$\beta = 0.017$
	2	0.28	0.26	$i = 17$
	3	0.02	0.04	
B	0	0.09	0.09	$\alpha = 0.041$
	1	0.62	0.63	$\beta = 0.018$
	2	0.28	0.25	$i = 17$
	3	0.01	0.03	

The region controlled extends from *ac* to *f*, and hence $N = 57$. The data for the two experiments are given in Table 1.

* The method of approximating the values of α, β, and i from the equations involved forcing a fit to the observed values for ranks 0 and 1, and then refining the values for ranks 2 and 3. Thus almost all the imprecision occurs in the last two values.

TABLE 4

Numerical estimates of α, β, and i from data from reversed acrocentric compound X chromosomes

Experiment	Rank	Observed frequency	Calculated frequency	Estimates of parameters
A	0	0.39	0.42	$\alpha = 0.050$
	1	0.17	0.21	$\beta = 0.013$
	2	0.44	0.37	$i = 11$
A + B	0	0.44	0.48	$\alpha = 0.048$
	1	0.12	0.20	$\beta = 0.011$
	2	0.44	0.32	$i = 11$

The estimate of N used here is 67. The data used are given in Table 2. (Ranks 2 and 3 are not distinguishable in these experiments and hence are combined).

normal and reversed acrocentric chromosomes, or it may be that the difference arises because the estimate of N is poor for the reversed acrocentric chromosome.

Predictions from the model

Since it is clear that, if this model is a reflection of some real physical situation, α is greater than β, and hence in a given region an exchange might be the third exchange in the tetrad more often than be the second. For example, let the chromosome be divided into three regions, 1, 2, and 3, and choose all tetrads with an exchange in region 2. Now we can compute the proportion which also has an ex-

change in region 3 when there was an exchange in region 1 and when there was not. According to the usual ideas about crossing over, these two proportions either ought be equal or, if an exchange in region 1 increases the interference between regions 2 and 3, an exchange in regions 3 (and 2) would be less frequent when there was an exchange in region 1 than when there was not. According to the assumptions used here, however, since α is greater than β, the contrary should obtain. Moreover, until this point we have not distinguished one end of the chromosome from the other, but now, if the physical basis of exchange starts more frequently from one end than the other, this comparison ought change depending on the direction on the chromosome that we use.

All such comparisons are subject to two technical limitations: (1) interference between regions 2 and 3 would tend to obscure any difference caused by the difference between α and β, and (2) the number of triple exchanges in specified regions is small, which will of course mean that the error on the ratios is high. For these reasons the following restrictions have been used: (1) region 3 must be at least 25 genetic units long, and (2) there must be at least 5 observed triple crossovers in the specified regions. With these limitations only two comparisons are possible, both of which come from experiment A (Table 1): in one case region 1 is the region from *sc* to *ec*, region 2 from *ec* to *cv*, and region 3 from *cv* to *f*; in the other case region 1 is from *f* to *g*, region 2 from *g* to *v*, and region 3 from *v* to *sc*. This is, fortunately, the same over-all region but proceeding in two different directions—the first from the tip to the centromere and the second from the centromere to the tip.

The precise comparison is

$$\frac{2,3}{2+2,3} \textit{ vs. } \frac{1,2,3}{1,2+1,2,3}.$$

Proceeding from the tip to the centromere, this comparison is 0.53 (2888/5490) *vs.* 0.71 (48/68). In the other direction it is 0.48 (2888/6068) *vs.* 0.24 (64/268). These numbers suggest that the proper direction is from the tip toward the centromere. It might be noted that this is what one would expect because the interstitial heterochromatin of the reversed acrocentric and reversed ring compounds, which we suppose to be the synaptic equivalent of the first exchange, is distal in these compounds.

A second prediction from this model suggests itself. If a given genetic region is moved from a proximal (i.e. near the centromere) position to a distal one, crossing over in that region ought to increase. This is because when a region is distal, the per-unit probability of an exchange in the region would most often be α, whereas the same region in a proximal position would (a good fraction of the time, at least) be so placed that the per-unit probability of an exchange would be β. That this prediction is realized experimentally in Drosophila has, of course, been known for many years and is generally termed the "centromere effect" (BEADLE 1932).

One final point should perhaps be made. The data presented here could very

likely be considered, with respect to this model, in other and perhaps more precise ways. It is thought, however, that owing to the imprecision of the available data—most particularly the data from the reversed acrocentric compounds, which are at best crude—such analyses are not justifiable at present.

SUMMARY

A model is proposed that attempts to rationalize the frequency distribution of tetrads by rank from free X chromosomes with that of reversed acrocentric and reversed ring compounds. The former distribution is characterized by a high frequency of tetrads of ranks 1 and 2 with a lower frequency of tetrads of ranks 0 and 3, whereas the latter distribution shows a high frequency of ranks 0 and 2, with a low frequency of tetrads of rank 1 (the frequency of tetrads of rank 3 or more is indeterminate).

The model proposed is built on the supposition that the physical basis of exchange (e.g., chromosome replication) starts at one end of the chromosome (some evidence presented here suggests that this is the distal end) and proceeds toward the other end with a per-unit probability of an exchange equal to α. After an exchange has occurred, however, this probability changes (say to β); a third exchange changes it back to α, a fourth exchange causes it to revert to β, and so on. For the reversed acrocentric and reversed ring compounds this same model applies except that the compounds are assumed to start the process with the β probability instead of the α probability. This is physically the same as supposing that the heterochromatic connection between the component chromosomes of the compound is the synaptic equivalent of the first exchange.

With some reasonable approximations to account for chiasma interference, for $0.04 < \alpha < 0.05$ and for $0.01 < \beta < 0.02$, the data from normal chromosomes and from the compound chromosomes are approximated.

It is suggested that this notion will account for certain peculiar relationships between exchanges manifest in data from normal X chromosomes in Drosophila (see text), and also can account for the well known "centromere effect" phenomenon.

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ESTIMATION OF THE COMPONENTS OF HETEROSIS

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IN previous papers (JINKS 1954, 1956) the analysis of the parents, F_1's, F_2's and backcross generations of an 8×8 diallel between inbred varieties of *Nicotiana rustica* using the method of diallel analysis described by JINKS and HAYMAN (1953), and extended by JINKS (1954), HAYMAN (1954), DICKINSON and JINKS (1956) and JINKS (1956) have been presented. They showed that nonallelic interactions, as well as additive and dominance effects, play an important role in the inheritance of all the three characters followed, namely, final height, time of flowering and leaf size. This finding was subsequently confirmed for a number of diallel sets of crosses in other species where a wide range of characters were followed (JINKS 1955; ALLARD 1956). What is more important to our present discussion, however, was the finding that the F_1 generation of crosses showing nonallelic interactions were in general superior in their performance to those of noninteracting crosses. This appears to implicate nonallelic interactions as a major source of heterosis, and we shall now attempt to assess the magnitude of their contribution relative to those of the other components of heterosis.

The components of heterosis

If we define heterosis as the difference between the mean of an F_1 family and that of its better parent, the expectations can be expressed in terms of the genetic parameters for additivity (d) and dominance (h) (MATHER 1949) and the nonallelic interaction components (i, j, and l) of HAYMAN and MATHER (1955) together with parameters expressing the degree of association or dispersion of the genes in the homozygous parents.

Consider first the case in which all genes have similar effects, and all pairs of genes similar interactions. Let the parents P_1 and P_2 differ at k loci, and of these let P_2 have k' of greater effect. Of the $\frac{1}{2}k\ (k-1)$ pairs of loci, $k'\ (k-k')$ are dispersed and the remainder associated. The mean parental phenotypes are then

$$\overline{\frac{P_1}{P_2}} = \pm (k\text{-}k')d \mp k'd + [\tfrac{1}{2}k(k\text{-}1) - k'(k\text{-}k')]i - k'(k\text{-}k')i \mp \tfrac{1}{2}(k\text{-}2k')\ (k\text{-}1)j + \tfrac{1}{8}\,k(k\text{-}1)l.$$

If, now, we write $r = 1 - \dfrac{2k'}{k}$

this becomes $\overline{\dfrac{P_1}{P_2}} = \pm r\Sigma d + \dfrac{kr^2-1}{k-1}\,\Sigma i \mp r.\Sigma\tfrac{1}{2}j + \Sigma\tfrac{1}{4}l.$

r may be taken as a measure of the degree of association for if $r = 1$, P_1 contains all the genes of greater effect, and if $r = -1$, P_2 contains all the genes of greater effect. In either case there is complete association. If on the other hand $r = 0$, $k' = \frac{1}{2}k$ so that the genes of greater effect are equally shared between P_1 and P_2, giving the maximum possible dispersion.

It is convenient to write the equation for the mean parental phenotype more shortly as

$$\overline{\frac{P_1}{P_2}} = \pm [d] + [i] \mp \tfrac{1}{2}[j] + \tfrac{1}{4}[l]$$

where $[d] = r\Sigma d$, $[i] = \dfrac{kr^2-1}{k-1}\Sigma i$, $[j] = r\Sigma j$, $[l] = \Sigma l$.

Writing in addition

$$[h] = \Sigma h$$

we have (c.f. HAYMAN and MATHER 1955 Table 3)

$$\overline{\frac{B_1}{B_2}} = \tfrac{1}{2}(\pm[d] + [h] + \tfrac{1}{2}[i]) \qquad \overline{F_2} = \tfrac{1}{2}[h]$$

$$\overline{F_3} = \tfrac{1}{4}([h] + \tfrac{1}{4}[l])$$

$$\overline{F_1} = [h] + \tfrac{1}{4}[l]$$

The scaling tests become

$$A = \overline{P_1} + \overline{F_1} - 2\overline{B_1} = \tfrac{1}{2}([i] - [j] + [l]). \; C = \overline{P_1} + \overline{P_2} + 2\overline{F_1} - 4\overline{F_2} = 2[i] + [l]$$

$$B = \overline{P_2} + \overline{F_1} - 2\overline{B_2} = \tfrac{1}{2}([i] + [j] + [l]). \; D = \overline{P_1} + \overline{P_2} + 2\overline{F_2} - 4\overline{F_3} = 2[i] + \tfrac{1}{4}[l]$$

The quantities $[d]$, $[h]$, $[i]$, $[j]$ and $[l]$ may be estimated using the following relations.

$$[d] = \overline{B_1} - \overline{B_2} \qquad [j] = 2\overline{B_1} - \overline{P_1} - 2\overline{B_2} + \overline{P_2}$$

$$[h] = \overline{F_1} - 4\overline{F_2} - \tfrac{1}{2}\overline{P_1} - \tfrac{1}{2}\overline{P_2} + 2\overline{B_1} + 2\overline{B_2}$$

$$[i] = 2\overline{B_1} + 2\overline{B_2} - 4\overline{F_2} \qquad [l] = \overline{P_1} + \overline{P_2} + 2\overline{F_1} + 4\overline{F_2} - 4\overline{B_1} - 4\overline{B_2}$$

The better parent is

$$\overline{P_0} = |\,[d] - \tfrac{1}{2}[j]\,| + [i] + \tfrac{1}{4}[l]$$

where $|\,[d] - \frac{1}{2}[j]\,|$ means the absolute value, without regard to sign, of $[d] - \frac{1}{2}[j]$. Hence measure of heterosis is

$$\begin{aligned}
\overline{F_1} - \overline{P_0} &= \; |\,[h] - [i]\,| - |\,[d] - \tfrac{1}{2}[j]\,| \\
&= \; [h] - [i] - [d] + \tfrac{1}{2}[j] \text{ if } [h] > [i] \text{ and } [d] > \tfrac{1}{2}[j] \\
&= \; [h] - [i] + [d] - \tfrac{1}{2}[j] \;\cdots\cdots\cdots\cdots\; [d] < \tfrac{1}{2}[j] \\
&= -[h] + [i] - [d] + \tfrac{1}{2}[j] \;\cdots\; [h] < [i] \;\cdots\; [d] > \tfrac{1}{2}[j] \\
&= -[h] + [i] + [d] - \tfrac{1}{2}[j] \;\cdots\cdots\cdots\cdots\; [d] < \tfrac{1}{2}[j]
\end{aligned}$$

When the condition that different loci shall have equal effects is relaxed, it becomes necessary to take account of the differences in effect when assessing the degree of association. A locus at which two possible allelomorphs had almost identical effects could clearly make only a small contribution to the effective degree of association or dispersion. However, all the above formulae remain valid except those connecting $[d]$, $[i]$ and $[j]$ with the basic model.

This is easily seen if we write $d_a^* = \pm d_a$ and $j_a \mid_b{}^* = \pm j_a \mid_b$ according as P_1 or P_2 carries the gene of greater average effect at the A locus, and $i_{ab} = \pm i_{ab}$ according as the genes at the loci A and B are associated or dispersed. Then

$$\overline{\frac{P_1}{P_2}} = \pm \Sigma d^* + \Sigma i^* \mp \tfrac{1}{2}\Sigma j^* + \tfrac{1}{4}\Sigma l$$

$$\overline{B}_1 = \tfrac{1}{2}(\Sigma d^* + \Sigma h) + \tfrac{1}{4}\Sigma i^*$$

etc., and the formulae are of the same form as those described above for the special case of equal genic effects, except that we now have

$[d] = \Sigma d^*$, $[h] = \Sigma h$, $[i] = \Sigma i^*$, $[j] = \Sigma j^*$ and $[l] = \Sigma l$.

An equivalent form is

$[d] = r_d \Sigma d$, $[i] = q_i \Sigma i$, $[j] = r_j \Sigma j$

where now r_d and r_j are the degree of association reckoned as before, except that each locus A is assigned a score equal to d_a or $\Sigma_b j_a|_b$ respectively, instead of simply 1, when the number of loci are being counted. Similarly, q_i is the proportion of associated pairs minus that of the dispersed pairs, the counting being carried out with scores equal to i. Since some of the i's and j's may be negative, q_i and r_j are not restricted to being less than 1 in absolute value.

Classification of nonallelic interactions

For two interacting genes the relationship between the parameters for nonallelic interactions and the classical interactions have been given by HAYMAN and MATHER (1955). For example, classical complementary genes are given by the relationship $d_a = d_b = h_a = h_b = 3/2$ $(i_{ab} = j_a|_b = j_b|_a = l_{ab})$ and duplicate genes by $d_a = d_b = h_a = h_b = -\tfrac{1}{2}$ $(i_{ab} = j_a|_b = j_b|_a = l_{ab})$ while other classical interactions can be derived from these by inequality of d_a and d_b.

The classical interaction can, however, be divided into two groups, those where the over-all effects of the nonallelic interactions reinforce the effects of dominance, e.g. complementary genes, recessive epistasis, etc., and those where the two effects are opposing, e.g., duplicate genes, dominant epistasis, etc. Where we are dealing with the sum or balance of the effects of many genes, inequalities in the contribution of individual genes (such as distinguish complementary genes and recessive epistasis or duplicate genes and dominant epistasis) would be difficult or impossible to detect: the only feature of the classical classification we can profitably utilize, therefore, is the distinction between reinforcing and opposing interactions. These will be referred to as complementary and duplicate type interactions respectively.

In practise the classification must primarily depend on the relative signs of

$[h]$ and $[l]$ as only these two components of the generation means are independent of the degree of association, which can itself take sign, and hence affect the sign of $[d]$, $[i]$ and $[j]$ independently of the sign of Σd, Σj and Σi.

OBSERVATIONS AND DISCUSSION

Nonallelic interactions and heterosis

The experimental design of the 8 × 8 diallel set of crosses between inbred varieties of *N. rustica* has been described in detail elsewhere. In general only the two seasons 1952 and 53, when parents, F_1's, F_2's, and backcrosses were grown simultaneously, concern us here.

For the data collected in both seasons the analysis of the family means for the character final height showed apparent overdominance and deviation of the W_r/V_r regression from a linear slope of 1, hence there was disagreement with the assumption of independence of the genes (JINKS 1954, 1956). The suggestion that this failure of the assumption was due to the dependence of the genes in their action (JINKS 1954) i.e. nonallelic interaction, was borne out by the scaling tests of MATHER (1949) and an analysis of the components of variation (JINKS 1956).

The A, B and C scaling tests of MATHER were combined into a joint test using the squared standard deviations of the generation means averaged over all crosses as weights (CAVALLI 1952). This joint test detected 10 crosses showing significant ($P < 0.05$) nonallelic interaction in 1952 and the same 10 plus 2 others in 1953 (JINKS 1956). However, while this test allowed for different nonheritable components of variation in different generations, it did not allow for differences between crosses for the magnitude of this component. Reanalyses have therefore been made for comparison, using the variance of the generation means appropriate to each individual cross. The degrees of freedom available for estimating the nonheritable components of each generation mean for a single cross in our experimental design are as follows:—

Degrees of freedom of the nonheritable components of variation

Generation mean	Reciprocal differences	Duplicate plots	Blocks	Recips. × blocks	Total degrees of freedom
$\overline{P_1}$ and $\overline{P_2}$	.	2*	1	.	3
$\overline{F_1}$	1	.	1	1	3
$\overline{F_2}$	1	4	1	1	7
$\overline{B_1}$ and $\overline{B_2}$	1	.	1	1	3

* Only 1 *df* available in 1952 making total for each parent $\overline{P_1}$ and $\overline{P_2}$ 2 *df*.

Since our comparisons will be between the means of plots, the degrees of freedom for the nonheritable components of variation are based on comparisons between replicate plot means of each generation grown in each season. Every plot, however, contains five plants, so that in all we have 20 plants of each parent (ten

in 1952), F_1 and backcross (B_1 and B_2) generation and 40 F_2 plants for each cross in each season.

The estimated variances and their degrees of freedom for the scaling tests are given by MATHER (1949). For example, the variance for the C scaling test is given by $V_C = V_{\overline{P}_1} + V_{\overline{P}_2} + 4V_{\overline{F}_1} + 16V_{\overline{F}_2}$ in our own case for 12 and 16 degrees of freedom in 1952 and 1953 respectively.

The same type of expectation holds for the components of the generation means. Thus the variance of $[i]$ is given by $V_{[i]} = 4V_{\overline{B}_1} + 4V_{\overline{B}_2} + 16V_{\overline{F}_2}$ for 13 degrees of freedom in both seasons.

Before, however, proceeding with the scaling tests and the estimation of the components of the generation means the sums of squares corresponding with the degrees of freedom listed above were estimated for each of the 28 crosses. These were pooled to give the variance of each of the generation means only if they were homogeneous.

A comparison of the crosses which show significant nonallelic interaction ($P < 0.05$) in these new tests and those which were significant in the earlier joint scaling tests in 1952 is given in Table 1.

All the ten crosses which showed nonallelic interactions on the joint scaling test show high significance for at least one of the individual scaling tests using errors appropriate to the generation means of individual crosses. In addition, four crosses which were not significant on the joint scaling test now show significance for one or other of the individual scaling tests.

Overall, therefore, 14 of the 28 crosses in 1952 give some indication of significant nonallelic interactions. For 1953 the overall total is 17 compared with 12 on

TABLE 1

The incidence of nonallelic interactions (+) as detected by the scaling tests for 1952

Cross	Scaling tests A	B	C	Joint scaling test (JINKS 1956)
1 × 2	+	+	+	+
1 × 4	—	—	+	+
1 × 8	—	—	+	+
2 × 3	+	+	—	+
2 × 4	—	+	+	—
2 × 5	+	—	—	—
2 × 6	+	—	—	+
2 × 8	—	+	+	+
3 × 4	—	—	+	+
3 × 8	+	+	—	+
4 × 6	+	—	+	+
4 × 8	+	—	+	+
5 × 6	+	—	—	—
6 × 8	+	—	—	—
Total	9	5	8	10

the earlier joint scaling tests. Of the 14 crosses in 1952 and the 17 in 1953 showing some indication of nonallelic interaction, 13 crosses are common to both seasons.

We can now make a four way classification of the 28 crosses in each of the two seasons, into interacters and noninteracters, and into those showing significant or no significant heterosis (Table 2).

TABLE 2

The incidence of heterosis and nonallelic interaction

Season	1952			1953		
	Interacters	Non interacters		Interacters	Non interacters	
Significant heterosis	8	1	9	11	4	15
No significant heterosis	6	13	19	6	7	13
	14	14	28	17	11	28

Taking both seasons together, there is a clear association between nonallelic interaction and heterosis, and the absence of nonallelic interaction and no heterosis ($\chi^2_{[1]} = 9.6$). This association is more marked in 1952 when the general level of heterosis was lower. Overall 60 percent of the interacting crosses show significant heterosis while only 20 percent of noninteracting crosses do so: or to put it another way, 80 percent of all heterotic crosses show significant nonallelic interactions.

This high correlation between the presence of nonallelic interaction and heterosis is even more marked when we consider the average heterosis of the crosses falling in each of these 4 classes. (Table 3)

TABLE 3

The incidence of nonallelic interactions and the average heterosis in inches

Season	1952		1953	
	Interacters	Non interacters	Interacters	Non interacters
Significant heterosis	8·81	5·60	13·68	5·03
No significant heterosis	1·49	0·02	1·24	0·37
Mean	5·40	0·61	9·02	2·32

If we now consider the seasons jointly, then 70 percent of the crosses showing nonallelic interactions also show significant heterosis. In fact only one cross which consistently shows nonallelic interaction consistently shows no significant heterosis.

While stressing this correlation between the incidence of nonallelic interaction and heterosis, it is obvious that heterosis can arise, albeit at a lower frequency, and with a lower mean expression, in the absence of significant nonallelic inter-

action, and further, that all crosses showing nonallelic interaction do not necessarily show heterosis.

Estimates of the components of the generation means

All six components of the generation means and their standard deviations have been estimated for all 28 crosses in both seasons. For those crosses where neither the components of nonallelic interaction nor any of the scaling tests are significantly different from zero, least squares estimates of the remaining components $[d]$ $[h]$ and M and their standard deviations have been obtained. Taking the latter crosses first, we find the following values for the additive $[d]$ and dominance $[h]$ components of the generation means (Table 4).

TABLE 4

Least squares estimates of the additive [d] *and dominance* [h] *components of the generation means for those cases showing no nonallelic interactions*

Cross	Season 1952 $[d]$	1952 $[h]$	1952 Heterosis	1953 $[d]$	1953 $[h]$	1953 Heterosis
1 × 3	2·6±2·0	8·7±3·8	5·6±1·4*	1·5±2·6	3·7±4·9	1·4±2·2
1 × 5	1·1±1·8	5·4±3·4	4·4±1·6	2·1±2·0	6·8±3·7	4·3±2·0
1 × 6	2·7±1·9	—0·6±3·6	—3·4±1·4	0·6±2·5	1·6±4·6	1·7±2·1
1 × 7	0·7±1·7	—0·4±3·2	—1·4±0·9	Significant nonallelic interaction		
2 × 7	2·1±3·1	4·0±5·8	2·1±2·6	9·1±2·4	2·7±4·5	—5·6±2·0
3 × 5	4·1±1·9	8·1±3·6	3·7±1·5	Significant nonallelic interaction		
3 × 6	5·5±2·3	3·1±4·3	—2·9±1·2	1·8±1·6	1·5±3·0	—1·3±1·3
3 × 7	3·6±1·5	3·6±2·9	—0·5±1·1	2·3±2·1	—0·8±3·9	—2·8±2·1
4 × 5	7·3±2·6	9·8±4·9	1·9±1·6	9·5±3·6	17·8±6·7	8·3±1·7*
4 × 7	8·0±1·7	6·4±3·1	—0·7±1·6	Significant nonallelic interaction		
5 × 6	Significant nonallelic interaction			2·4±1·9	6·7±3·6	3·8±1·5
5 × 7	1·1±1·2	0·8±3·7	0·5±1·3	2·6±1·9	7·0±3·7	4·5±1·9
5 × 8	1·9±1·7	3·0±3·3	1·9±1·7	Significant nonallelic interaction		
6 × 7	2·2±1·7	—2·9±3·3	—4·2±1·4	4·9±1·8	8·0±3·3	3·6±1·1*
6 × 8	Significant nonallelic interaction			2·3±1·5	5·1±2·9	3·6±0·7*
7 × 8	0·9±1·5	—1·4±2·8	—2·2±1·7	Significant nonallelic interaction		

* Significant heterosis at $P \leqslant 0{\cdot}05$.

None of the crosses in this table, i.e. the crosses which show no significant nonallelic interaction, show significant heterosis in both seasons. Three crosses 1 × 3, 4 × 5, and 6 × 7, however, do show significant heterosis in one or other of the two seasons without any suggestion of nonallelic interaction in either season. We must, therefore, consider the possibility that the significant heterosis in these crosses is the result of overdominance or the dispersion of dominant increasing alleles in the parental lines. In these three crosses the dominance component of the generation means $[h]$ varies from 1·6 to 3·3 times the value of the additive component $[d]$. It is clear that this situation could arise as a result of overdominance ($\Sigma h > \Sigma d$), incomplete association of dominant increasing alleles in the parental lines ($r_d < \pm 1$), or any combination of these two. Should, however, over-

dominance be responsible for the heterosis, then the dominance (H) component of variation should be greater than the additive (D). Estimates of these two components of variation for the three crosses 1×3, 4×5 and 6×7 using the F_2 and backcross variances (see MATHER 1949) give no indication of overdominance. An explanation based on dispersion of the dominant increasing alleles would, therefore, appear to be more in line with the observations.

The remaining consistently noninteracting crosses listed in Table 4 show no significant heterosis and hence require no further comment.

The interacting crosses, as we have seen, fall into two groups, according to whether or not they show significant heterosis. Examples of interacting crosses which show no significant heterosis are provided by 1×7, 1×8, 3×8, 5×6, 5×8, and examples of the estimates of the components of their generation means are listed in Table 5. The standard errors of these components are obtained from those of the generation means from which the components are estimated.

TABLE 5

Estimates of the components of the generation means and of heterosis for a number of crosses which show significant nonallelic interaction but no heterosis

Cross and season	3×8 (1952)	5×6 (1952)	3×4 (1952)	1×7 (1953)
Components of generation means				
$[d]$	5·40± 0·94*	—2·95± 0·77*	—1·65± 3·56	5·76± 2·18*
$[h]$	28·70± 3·30*	9·80± 4·11*	—1·60± 9·51	—18·01± 7·46*
$[i]$	23·90± 2·51*	6·50± 2·80*	—17·80± 8·11*	—25·38± 5·62*
$[l]$	—44·70± 4·64*	71·20± 4·76*	15·50±15·52	47·18±11·07*
$[j]$	4·90± 2·61	—9·60± 2·34*	—27·20± 7·24*	1·54± 4·48
M	17·63± 1·36*	33·95± 1·60*	53·28± 4·23*	58·97± 3·16*
Heterosis	1·85± 1·62	1·45± 1·29	4·25± 2·65	2·47± 3·16
Components of heterosis				
$[h]$	28·70	9·80	—1·60	—18·01
$-[d]$	—5·40	2·95	1·65	—5·67
$\frac{1}{2}[j]$	2·45	—4·80	—13·60	0·77
$-[i]$	—23·90	—6·50	17·80	25·38
Total	1·85	1·45	4·25	2·47

* Significant at $P < 0{\cdot}05$.

The four examples in Table 5 are typical of the situations that have been found in nonheterotic interacting crosses. In each of these crosses internal cancellation of the contributions of the four components of heterosis leads to an overall non-significant heterosis, although the individual components are in general highly significant.

In three of these crosses $[l]$ and $[h]$ have opposite signs suggesting duplicate type interactions. In cross 3×4, however, neither $[l]$ nor $[h]$ are significantly different from zero. Apart from the small contributions of $[d]$ and $\frac{1}{2}[j]$ it is the opposition of $[i]$ and $[h]$ which leads to the absence of heterosis in crosses 3×8,

5×6 and 1×7. In crosses 3×8 and 5×6, the positive contribution of $[h]$ is balanced by the negative one of $[i]$ while in cross 1×7 it is the reverse. Furthermore the nonsignificant $[j]$ component in crosses 3×8 and 1×7 suggest that either r_j, Σj or both are very small or zero. If these interactions are in fact comparable with the duplicate types of classical genetics then it is r_j which is small or zero, i.e. there is dispersion of the interacting genes in the parental lines. This would square with the sign of $[i]$ relative to $[h]$ which suggests that q_i is negative. Overall, therefore, these interactions are compatible with dispersed duplicates.

Contrasting with this situation we have cross 3×4 where the absence of heterosis results from the opposition of the contributions of $[i]$ and $\frac{1}{2}[j]$. Clearly r_j cannot be very small or zero, i.e. there is association of the interacting genes in the parental lines. The insignificance of $[h]$ and $[l]$ make them doubtful indicators of the type of interactions involved. Since neither classical complementary nor duplicate genes in association give heterosis in the F_1, comparison with classical models provide no further clues as to the type of interaction involved in this cross.

Thus in the crosses which show nonallelic interactions but no heterosis, we find either opposition between the dominance and interaction components of heterosis, i.e. duplicate genes or association of the interacting genes in the parental lines.

Cross 1×4 may be taken as typical of many which show both heterosis and nonallelic interaction. It has been grown in every season from 1951 to 1956 (We are indebted to Dr. E. L. Breese for the observations in 1955 and 1956). From 1952 to 1956 F_2's were included along with the parents and F_1 generations, while in 1952 and 1953 backcrosses were included and in 1956 one of the backcrosses $(1 \times 4) \times 1$ and $(4 \times 1) \times 1$ was grown. All the components of the generation means cannot be estimated in all the seasons, but estimates of certain combinations can be obtained from most of them (Table 6).

All estimates in Table 6 apart from the first and second in 1953 are significant at $P \ll 0.05$. This low significance in 1953 can be traced to the large reciprocal cross and block differences, but apart from this there is a general consistency in sign and magnitude of the compound components of the generation means and heterosis over all seasons. Clearly the interpretation arrived at from a complete

TABLE 6

The components of the generation means for cross 1 × 4

	Season	1951	1952	1953	1954	1955	1956	Mean
Components	$2[i]+[l]$	....	—17·08	—0·66	—11·98	—10·66	—32·52	
	$[i]+[j]$	....	—29·88	—2·76	....	....	—24·41	
Components of heterosis	$[h]-[i]$	21·40	20·45	29·01	20·04	18·46	17·18	21·09
	$-[d]+\frac{1}{2}[j]$	—7·20	—8·90	—7·86	—3·31	—10·75	—4·86	—7·15
Heterosis		14·20	11·55	21·15	16·73	7·72	12·32	13·94

analysis in 1952 will not differ in detail from that which would apply in the other seasons.

In 1952 the estimated components of heterosis are as follows:

Component	$[h]$	$-$	$[i]$	$-$	$[d]$	$+ \frac{1}{2}[j]$	$=$	Heterosis
Estimate	4·25	+	16·20	−	2·05	− 6·85	=	11·55
Compound estimate from Table 6.	20·45			−	8·90			

Clearly heterosis in this cross results from reinforcing dominance and non-allelic interactions; the latter making the major contribution, and apart from 1953 where the nonsignificance of many components makes interpretation difficult, this situation appears to hold in the other seasons.

The closest comparable classical interaction to that found in cross 1 × 4 is, of course, a complementary type with the interacting genes preponderantly dispersed in the parental lines.

Most interacting crosses showing heterosis are of this type, but contrasting with them is a situation which is at its most extreme in cross 1 × 2. The components of the generation means of the latter are listed in Table 7.

For 1953 the two blocks are given separately as the generation means differed significantly more between blocks than did duplicate or reciprocal plots within a block.

In 1952 there is apparently complete dispersion of duplicate genes, the heterosis resulting from the excess of the positive dominance $[h]$ over the negative interaction $[i]$ contribution. In block I of 1953 the situation is comparable with cross 1 × 4 (Table 6) i.e. a complementary type interaction with the interacting genes dispersed in the parental lines, the heterosis resulting from the interaction component $[i]$. Block II in the same season, however, is intermediate; the significant $[j]$ component suggesting association. In the absence of a significant $[l]$ either a duplicate or complementary type of interaction would equally well fit the data.

TABLE 7

The components of the generation means for the cross 1 × 2

Season	1952	1953 I	1953 II
Components			
$[d]$	0·00± 2·45	2·00± 2·69	−2·70± 0·61*
$[h]$	53·19± 9·89*	4·58±13·15	22·05± 6·45*
$[i]$	38·10± 7·43*	−19·40± 8·78*	2·40± 5·17
$[l]$	−60·72±11·89*	51·45±14·12*	−11·10± 8·53
$[j]$	−4·80± 5·16	−1·95± 5·94	−14·70± 3·42*
M	12·98± 3·83*	60·68± 6·04*	54·77± 4·15*
Heterosis	12·69± 1·97*	21·00± 3·81*	15·00± 3·01*

* $P < 0·05$.

Clearly the genotype environmental interaction in this cross is such that a consistent interpretation of the basis of the heterosis is impossible. We can, however, profitably examine the nature of this interaction.

Between the three environments the magnitude of the heterosis in cross 1 × 2 is positively correlated with the contribution of $[i]$, and negatively correlated with the contribution of $[h]$ to the heterosis. Superficially, therefore, in those environments which allow a fuller expression of the heterotic potential of this cross, nonallelic interactions play the major role in its production. We can now enquire as to the nature of an environmental restriction to the development of heterosis which would lead to a change from a complementary to a duplicate type of nonallelic interaction.

An environmental restriction to the development of the full height potential of the parents and offspring of cross 1 × 2 would effect the F_1 more than any other generation. Similarly, the backcross to the larger parent would be affected more than that to the smaller parent. If we impose such restrictions on a classical complementary system, sooner or later, we obtain estimates of the generation means which resemble those obtained from a duplicate or intermediate type of interaction. Furthermore, changes of scale e.g. antilogs, lead to the same type of change. Environmental restrictions on the attainment of the full height potential, therefore, provide an adequate explanation of the apparent inconsistency of the genetical basis of heterosis in cross 1 × 2.

CONCLUSIONS

Heterosis is a complex genetical phenomenon depending on the balance of the additive, dominance and interaction between homozygous/homozygotes and homozygous/heterozygous components of the generation means as well as on the distribution of the genes in the parental lines. Strangely enough it does not depend on the interaction between heterozygous/heterozygous combinations, but not so strange when we remember that heterosis is but the reverse counterpart of inbreeding depression.

As a consequence of its complexity the presence or absence of heterosis is not in itself indicative of the presence or absence of any particular type of gene action or interaction; it can result from a whole range of combinations of gene effects, many of which have been illustrated by our *N. rustica* data. Nevertheless, there is a correlation between the presence or absence of heterosis and the presence or absence of nonallelic interactions, and while heterosis can arise in the absence of nonallelic interactions it does so with a lower frequency and a lower mean expression.

SUMMARY

Heterosis can be expressed in terms of four of the components of the generation means of a cross between two inbred lines. These components can be estimated from the means of the parents, F_1's and F_2's and backcross generations of such a cross. This allows an assessment of the relative roles of dominance, nonallelic interaction and gene distribution in the production of heterosis and also a

preliminary classification of the interaction into a complementary or duplicate type.

The method is illustrated by an analysis of the components of heterosis for final height in 28 crosses between inbred varieties of *N. rustica*.

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PHENOTYPIC PROPERTIES OF HETEROZYGOTES IN THE BACTERIOPHAGE T4[1, 2]

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THE *r*II region of the genome of the bacteriophage T4 has been particularly useful for the investigation of mutation (BENZER 1955, 1957), recombination (CHASE and DOERMANN 1958), and gene function (KRIEG 1957). This is largely due to the differential response of phage mutant and nonmutant in this region to the bacterium *Escherichia coli* K12 (λ). While *r*II$^+$ phage may infect K12 (λ) with a subsequent liberation of progeny, *r*II phage absorb to and kill K12 (λ), but the complex does not produce any infectious progeny.

By using mixed infections with different combinations of phage genotypes, BENZER (1955) was able to show that the *r*II region of the T4 genome consists of two adjacent but autonomously functioning segments. These segments he designated the A and B cistrons (BENZER 1957). He found that K12 (λ) releases no phage when simultaneously infected with two different *r*A or with two different *r*B mutants, whereas infections of K12 (λ) with both an *r*A and an *r*B mutant do result in the production of phage progeny (BENZER 1955).

It therefore appears that both an unmutated A cistron and an unmutated B cistron must be present in an infected K12 (λ) bacterium for the production of mature phage progeny. Since the genetic material of phage heterozygotes might have a structural organization differing from the presumed "diploidy" of a mixed infection, it is of interest to know if both structural conditions display similar phenotypic properties.

From crosses of T2 involving a variety of markers, HERSHEY and CHASE (1951) found that two percent of the progeny particles recovered were heterozygous, i.e., showed subsequent segregation for a given pair of alleles. A heterozygote rarely segregates for more than one pair of alleles, except in cases of close linkage. Heterozygous *r*II markers in T4 also show this type of segregation pattern, and likewise are recognizably diploid only for short regions of the genome (DOERMANN and KATAJA 1957). LEVINTHAL (1954) has presented evidence that the heterozygotes are intermediate stages in recombinant formation and that they are

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usually recombinant for regions on either side of the heterozygous, or "overlap," region. The rest of the genome may be either hemizygous (Figure 1a), or diploid but homozygous (Figure 1b). These two alternative models are indistinguishable on the basis of the existing data. The most probable kinds of heterozygotes arising in crosses involving two closely linked *r*II markers are depicted in Figure 2. The functional properties, with respect to growth in K12 (λ), of two of these types, the single, or r^+ recombinant heterozygote (Figure 2a) and the nonrecombinant heterozygote (Figure 2c) are examined in this paper.

LEVINTHAL (1954) suggested that the phage genome might be little more than a DNA molecule and that in a heterozygous region, the two complementary chains (WATSON and CRICK 1953) may be derived from different parents. If this were true, it might be imagined that heterozygotes represent an association more intimate than that afforded by two homologous chromosomes within a nucleus, and might display phenotypic properties differing from those of corresponding mixed infections.

However evidence will be presented in this paper which indicates that heterozygotes display phenotypic properties similar to those of corresponding mixed infections. The bearing of these results on the analysis of recombination and on the structure and phenotypic action of the hereditary material of phage will be discussed.

MATERIALS AND METHODS

Two strains of bacteriophage T4 were used in these experiments, T4D (DOERMANN and HILL 1953) and T4B (BENZER 1955). It was found that progeny from

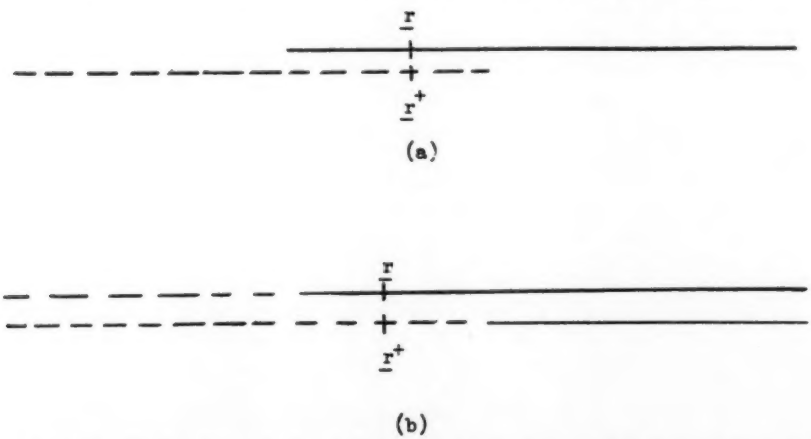

FIGURE 1.—The "partial replica" and "diploid" models for the structure of heterozygotes. Alternative models of the nature of heterozygotes, obtained from a cross of *r* (solid line) × *r*+ (interrupted line) are depicted. Interrupted and solid lines are to indicate the source of the genetic material derived from one parental type or the other. Type (a) will be referred to as the "partial replica" model and type (b) as the "diploid" model.

crosses of the type T4D rII$^+$ × T4B rII exhibited the segregation of at least three unidentified genetic characters, and therefore subsequent crosses were made only within, and not between, strains.

The r^+ and various rII mutants of T4B used were obtained from BENZER. These mutants are identified with numbers greater than 100, e.g., r_{145}. The relative map locations of their markers have been given by CHASE and DOERMANN (1958). The T4D r^+ and rII mutants r_{47}, r_{43}, and r_{51} were obtained from MISS M. CHASE. The mutant r_{73} was obtained from G. STREISINGER. The rest of the T4D rII mutants were all isolated from separate T4D r^+ plaques and all identified with numbers less than 100, e.g., r_{64}. With the exception of r_{51}, all the rII mutants used in this study have reversion indices of less than 10^{-5}. The reversion index of r_{51} is 10^{-4}. (The reversion index is the proportion of r^+ revertants in an rII stock, measured by plating on K12 (λ)). Less than 10^{-8} reversions were present in stocks of r_{168}, r_{145}, r_{47}, r_{61}, r_{64} and r_{73}. These mutants are apparently stable, but, with the exception of r_{47} (BENZER 1957), have shown no unusual behavior in crosses. The relative genetic locations of the markers of the T4D rII mutants, as determined by the present worker, are given in Figure 3 and more completely by

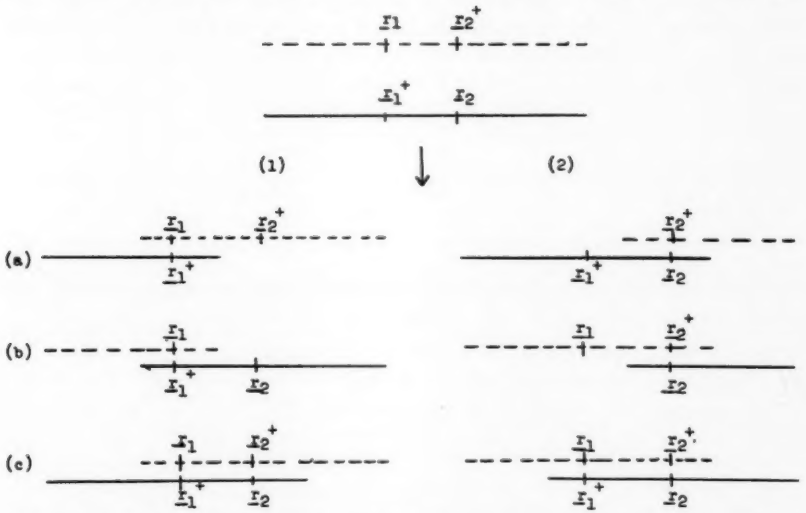

FIGURE 2.—Types of heterozygotes from $r \times r$ crosses. A two-factor cross, $r_1 \times r_2$, will give rise mainly to the three types of heterozygotes depicted in (a), (b), and (c). The $r+$ recombinant heterozygote (a) will give $r-+$ and either parental type, r_1 [as in (a)1] or r_2 [as in (a)2] as primary segregants. The r_1r_2 recombinant heterozygote (b) will give the r_1r_2 recombinant and and either parental type as the primary segregants. The nonrecombinants heterozygotes (c) produce the two parental types as primary segregants. The two types of recombinant heterozygotes (a and b) are formed as a consequence of the overlap including one but not both of the marked loci. The nonrecombinant heterozygotes only arise when the overlap region includes both marked loci.

EDGAR (1958). Some of these mutants carried the additional marker tu_{41} (DOERMANN and HILL 1953). This marker did not interfere with the scoring of mottled plaques (plaques containing both *r* and r^+ phage). No detectable differences between any of the *r*II mutants used in this study were noted with regard to the transmission coefficient, which under the conditions employed, was characteristically between 10^{-4} and 5×10^{-3}. (The transmission coefficient is the proportion of *r*II-infected K12 (λ) complexes which yielded progeny (BENZER 1955)).

Strain B of *Escherichia coli* was used as host for crosses. The plating bacteria were saturated overnight cultures of *E. coli* strain S/6, a derivative of B (DOERMANN and HILL 1953). The selective indicator used was *E. coli* strain 112–12 (λ *h*) No. 3, a derivative (WOLLMAN 1953) of K12, hereinafter called K. This strain was obtained from F. W. STAHL. It was used, in preference to K12 (λ *l*) used by CHASE and DOERMANN (1958), as it gives a lower transmission coefficient. This was advantageous in the experiments to be described.

The media used and the procedure for preparing phage stocks were those described by CHASE and DOERMANN (1958).

The crosses considered in this study were performed as described by CHASE and DOERMANN (1958). In addition, however, in order to obtain a high titer stock of progeny phage, the infected bacteria were diluted 100-fold into H-broth prior to serum treatment and allowed to lyse. The total progeny phage titer of such a lysate was usually about 2×10^8. As mentioned by CHASE and DOERMANN, the proportion of recombinants in such a lysate was often as much as a factor

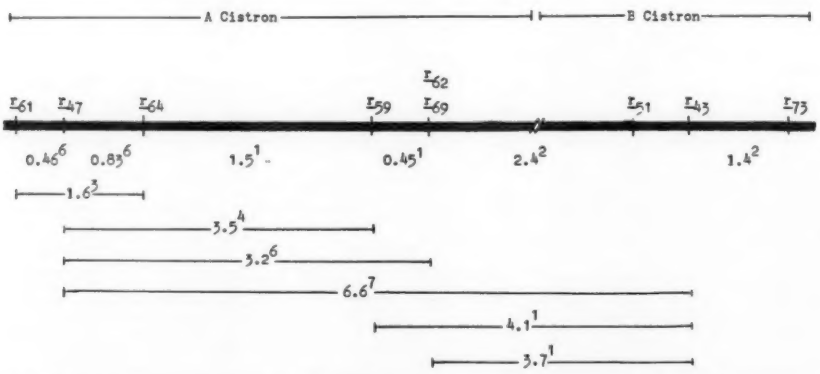

FIGURE 3.—A map of the *r*II region of T4D giving the markers and intervals referred to in this paper. The recombination values are calculated as twice the titer of progeny determined by plating on K divided by the titer of progeny obtained on S/6. The efficiency of plating on K relative to S/6 is usually about 0.8, and no correction is made for this factor. The superscript to the recombination values for a given interval indicates the number of crosses of which the given value is the mean. The standard deviation of these values was usually less than 20 percent of the mean. Other crosses with different *r*II markers (EDGAR 1958), not given in the figure, were also used to determine the best order of the markers.

of two greater than that in a more dilute lysate. The cause of this discrepancy is unknown.

The addition of chloroform to lysates as a precautionary measure to destroy unlysed complexes did not appear to influence the results to be discussed, and therefore its use was omitted.

Examination was made, by plating on S/6, of progeny and of progeny-K complexes (infective complexes formed by adsorption of the progeny to actively growing K). The procedure for preparing the progeny-K complexes was as follows. A growing culture of K was concentrated by centrifugation and resuspended in H-broth to a titer of between 2×10^8 and 8×10^8 cells/ml. Cyanide was added to give a final molarity of 0.005. Phage were added to this bacterial suspension such that the multiplicity of infection was less than 0.05. A ten minute adsorption period (in which over 95 percent of the phage were adsorbed) followed. At least 90 percent of the remaining unadsorbed phage were inactivated with anti-T4 rabbit serum. The infected bacteria were then diluted, and aliquots were plated on S/6.

Plaques on S/6 from direct plating of the progeny and from progeny-K complexes were scored as to whether they were phenotypically r, r^+, or mottled. The last type of plaque is made by infected bacteria which produce enough of both r and r^+ particles to give a plaque a mottled appearance. These may be produced either by heterozygotes or by bacteria mixedly infected with r and r^+ phage. Mottled plaques from the direct plating of the progeny are produced only by heterozygotes. The progeny-K complexes, on the other hand, depending upon the multiplicity of infection, produce a certain fraction of mottled plaques from cells mixedly infected with r and r^+ phage. Non-Poisson adsorption was found with K grown in H-broth. The extent of the deviation from random expectation was found to vary from day to day, but mixedly infected cells in control cultures, like the experimental tests in regard to multiplicity, usually occurred with a frequency twice the Poisson expectation. For this reason, the correction made for mixedly infected cells in Tables 1b, and 2 are, in all cases, two times the Poisson expectation. The multiplicities of infection in the experiments given in Table 1a were sufficiently low that no correction for mixedly infected cells was needed.

Since it was found that mottled plaques were misidentified if as many as 10^6 uninfected K bacteria were put on the plate together with the progeny-K complexes, very low multiplicities were not used in most experiments. The use of very low multiplicities necessitates fewer dilution steps from the adsorption tube, and thus a larger number of uninfected bacteria would be delivered to the plate.

It was found that mottling was most easily distinguished if the plates were incubated for 16 to 20 hours at 37° C. Therefore all plates were scored at this time.

RESULTS

Since r^+ particles may grow successfully in K bacteria, whereas r do not, the relative frequencies of r^+ to mottled plaques on S/6, with and without prior adsorption to K, will reveal whether mottling particles from a given cross are lost

by passage through K. The ratio used is the fraction of plaques containing both r^+ and r (in other words, mottled plaques) to the sum of r^+ and mottled plaques. For the progeny-K complexes in some experiments, since multicomplexes are present, this ratio must be corrected by estimating, as indicated in the materials and methods section, the fraction of mottled plaques produced by mixedly infected bacteria. Such plaques may then be reclassified as r^+.

Single heterozygotes from rII × rII⁺ crosses

Table 1a presents data on the growth in K bacteria of single heterozygotes from *r*II × *r*II⁺ crosses. From the χ^2 test for homogeneity, it may be seen that no loss of mottling particles from either the *r*IIA × *r*IIA⁺ ($r_{205} \times r^+$) or the *r*IIB × *r*IIB⁺ ($r_{43} \times r^+$) cross is detected after passage through K. The application of the methods of error analysis to the data in Table 1a (MARGENAU and MURPHY 1943) suggests that most, if not all, of the mottling particles function in K as *r*II⁺ particles, i.e., produce progeny.

Heterozygotes, if not detected as mottled plaques, will be scored as either r or r^+ plaques. Variability is observed in the appearance of mottled plaques which might suggest such misclassification. An estimate of the magnitude of misclassification of single heterozygotes as r plaques can be made. Very few phenotypically r plaques were observed among the plaques obtained from the progeny-K complexes of the above mentioned crosses. The r plaques probably represent transmission complexes. They give, however, an upper limit estimate of the proportion of heterozygotes which can grow in K bacteria but which are scored as r. This limit is less than nine percent in the $r_{205} \times r^+$ cross and less than five percent in the $r_{43} \times r^+$ cross.

Since there is no apparent reason why heterozygotes would be more likely misclassified as r^+ than as r, it is reasonable to assume that virtually all single hetero-

TABLE 1a

Progeny and progeny-K complexes from rII × rII⁺ crosses

Cross type	Cross	Method of anal.	No. plaques examined: mottled	r^+	r	Obs. ratio*	p value of χ^2	Efficiency of growth of mottling particles in K
		P	149	5768	6262	0.025		
*r*A × r^+	$r_{205} \times r^+$	P-K	297	11882	26	0.024	0.8>p>0.7	0.97±0.09
		P	137	4975	4799	0.027		
*r*B × r^+	$r_{43} \times r^+$	P-K	113	4290	5	0.026	0.8>p>0.7	0.96±0.12

* Mottled/mottled plus r^+.

Tables 1a and 1b. For all crosses tabulated, the plaques counted are given for both the progeny (P) and the progeny-K complexes (P-K). The "cross type" indicates the cistrons in which the markers are situated. The ratio calculated from the raw data is the ratio of mottled plaques to the sum of mottled plaques and r^+ plaques. In Table 1a, the p value of a χ^2 test for the homogeneity of the data is given. The last column of Table 1a gives the value obtained by dividing the P-K ratio by the P ratio, together with its probable error, determined by the method suggested by MARGENAU and MURPHY (1943), page 498. This value is an estimate of the fraction of heterozygotes which grow in K (see text). In Table 1b, the corrected ratios are calculated from the observed ratios corrected as indicated in the text. The map distances (last column) are taken from Figure 3, with the exception of the cross $r_{148} \times r_{168}$, which is given by CHASE and DOERMANN (1958).

zygotes are scored as mottled plaques. With this assumption, these experiments permit the conclusion that most, if not all, single heterozygotes have an *r*II$^+$ phenotype in K.

Crosses involving two r*II markers*

As will be seen from Figure 2, two types (a) and (c) of heterozygotes neither homozygous nor hemizygous for either of the *r* markers, should be formed in crosses involving two different *r* markers. These are the *r*$^+$ recombinant heterozygote (a) and the nonrecombinant heterozygote (c). The relative frequencies of these two types among the progeny should depend upon the distance between the two markers employed. As will be seen, the *r*$^+$ recombinant heterozygotes are probably a constant fraction of the *r*$^+$ recombinants, independent of the interval between markers, and therefore a diminishing fraction of the population as the distance between the markers becomes shorter. The nonrecombinant heterozygotes, on the other hand, should be an increasing fraction of the population as the distance between the markers is reduced, since it will be increasingly likely that the overlap region will include both markers. Preliminary experimental evidence supports the correctness of this notion (DOERMANN and KATAJA 1957). This fraction should approach the frequency of single heterozygotes in T4, which, from the data in Table 1a, is a little over one percent.

The *r*$^+$ recombinant heterozygotes (a, in Figure 2) should give mottled plaques due to the segregation of the *r* and *r*$^+$ markers, and, since they are similar in structure to the heterozygous progeny from *r*II × *r*II$^+$ crosses, should give an *r*II$^+$ phenotype in K bacteria.

The nonrecombinant heterozygotes (c, in Figure 2) could give mottled plaques due to the formation of *r*$^+$ recombinants as well as the regular *r* segregants. If even a small proportion of the nonrecombinant heterozygotes produce mottled plaques, it should be possible to determine whether they can grow in K bacteria, for, with closely linked *r* markers, the nonrecombinant heterozygotes should be more frequent than the *r*$^+$ recombinant heterozygotes, and their loss in K should be detected as a relative loss in mottling plaques from the progeny-K complexes compared to the direct plating of the progeny.

The results from experiments of this type are given in Table 1b and are graphically represented in Figure 4. It is seen that the crosses may be distinguished on the basis of whether or not a loss of mottling particles is observed in the progeny-K complexes.

Two-factor crosses involving r*II markers in the same cistron*

From crosses of two *r*'s, both of which are within the A or both within the B cistron, it appears that two kinds of mottling particles are produced, which may be differentiated by their ability or inability to produce progeny upon infection of K hosts. Since the *r*$^+$ recombinant heterozygotes should grow in K, the type unable to grow in K must be the nonrecombinant heterozygote. It will be noted from Figure 4 that these particles represent an increasing fraction of the progeny rela-

tive to the r^+ recombinants, as the map distance becomes shorter. This is to be expected of the nonrecombinant heterozygotes on the basis of the observations of DOERMANN and KATAJA (1957) cited earlier.

Table 2 presents data for progeny-K complexes from repeated crosses involving various pairs of *r*IIA markers and a repeated cross of a distantly linked *r*IIA and *r*IIB pair. An analysis of variance was performed on the corrected ratios (mottled to mottled plus r^+) from these crosses. The corrections were made as indicated in the section on materials and methods. It was found that the variance among repeated crosses was sufficiently great to mask differences, if any, for this ratio between different pairs of markers. These data are thus not inconsistent with the notion that the r^+ recombinant heterozygotes represent a constant fraction of the r^+ recombinants, over the range of map distances employed.

The following observations show that the nonrecombinant heterozygotes probably have no greater chance of growing in K bacteria than do *r* particles. They therefore probably possess an *r*II phenotype in K. The progeny from the cross $r_{227}r_{320} \times r_{205}$ were adsorbed to K as described previously, except that the cells were not exposed to cyanide. The infected complexes were then superinfected with $T6r_{47}$ (the r_{47} marker of T4, introduced into T6) at fifteen minutes and plated on S/6. The superinfection with $T6r_{47}$ was performed to sterilize the uninfected K bacteria which in this experiment were in sufficient numbers, if allowed to grow on the plate, to obscure recognition of the phenotypes of the plaques. From

TABLE 1b

Progeny and progeny-K complexes from rII × rII crosses

Cross type	Cross	Method of analysis	No. plaques examined			Observed ratio mottled/ mot. plus r^+	Corrected ratio	Map distance
			mottled	r^+	r			
$rB \times rB$	$r_{43} \times r_{73}$	P	57	95	9522	0.38	...	1.6
		P-K	214	1042	11	0.17	0.12	
$rA \times rA$	$r_{145} \times r_{168}$	P	8	10	13705	0.44	...	0.14
		P-K	59	377	.. †	0.14	0.08	
	$r_{47}tu_{41} \times r_{61}$	P	22	25	9342	0.47	...	0.46
		P-K	154	896	.. †	0.15	0.12	
	$r_{61} \times r_{64}$	P	26	40	4975	0.39	...	1.6
		P-K	54	672	.. †	0.07	0.04	
	$r_{47}tu_{41} \times r_{59}$	P	36	194	9550	0.16	...	3.5
		P-K	144	1360	....	0.10	0.07	
$rA \times rB$	$r_{69} \times r_{51}$	P	46	61	8050	0.43	...	2.4
		P-K	184	236	.. †	0.44	0.39	
	$r_{69} \times r_{43}$	P	34	44	3178	0.44	...	3.7
		P-K	307	555	.. †	0.36	0.35	
	$r_{59} \times r_{43}$	P	90	202	11310	0.31	...	4.1
		P-K	420	1105	.. †	0.28	0.25	
	$r_{47}tu_{41} \times r_{43}$	P	62	258	9533	0.19	...	6.6
		P-K	126	653	.. †	0.16	0.13	

† *r* plaques were observed but are not tabulated.

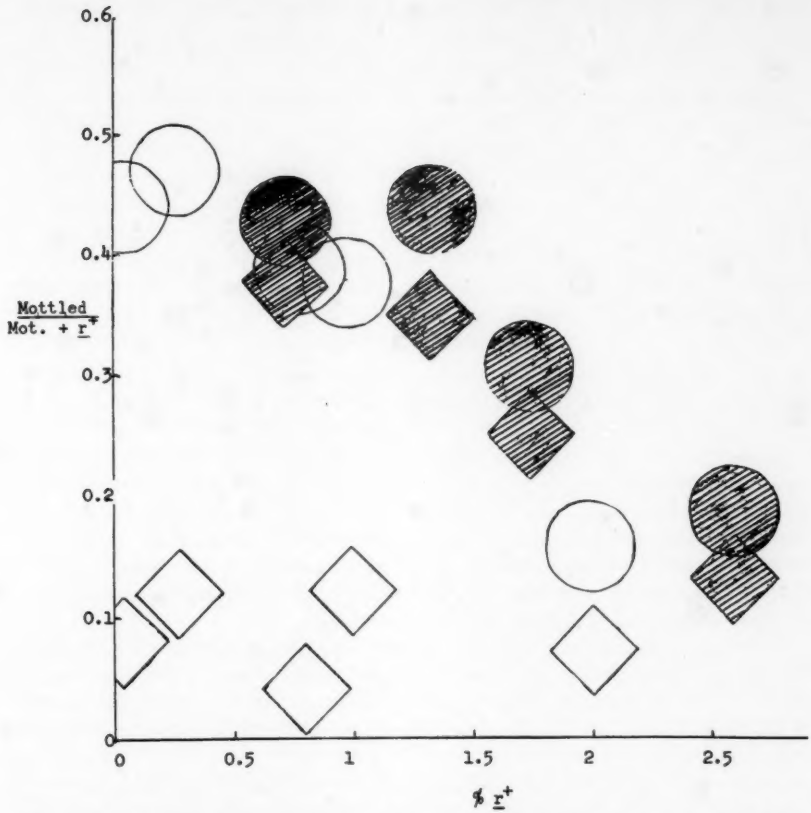

FIGURE 4.—A graphic representation of the results given in Table 1b. The ratio, mottled to mottled plus $r+$, of the progeny and progeny-K complexes, is plotted against the percent $r+$, calculated from the direct plating of the progeny, for each cross. For every circle, which represents progeny plated directly on S/6, there is a corresponding diamond, which represents the corrected ratio from the progeny-K complexes. The shaded symbols represent crosses involving two markers in different cistrons (rA $\times$ rB). The white symbols signify crosses of two markers within the same cistron (rA $\times$ rA, or rB $\times$ rB).

controls it appeared that after superinfection at this late time most of the T6r_{47} are excluded from the infected cells. The markers used are in the A cistron in the order r_{227}, r_{205}, r_{320} and are very closely linked, such that close to one percent nonrecombinant heterozygotes will be formed. Of the progeny-K complexes, about 0.01 percent gave $r+$ and mottled plaques, and 0.01 percent produced r plaques. Of the former, one third were mottled. However, the relative frequencies of $r+$ and heterozygotes cannot be determined reliably, since some of the mottled

TABLE 2

Repeated determinations of the ratio of mottled to mottled plus r+ plaques for various pairs of rII markers

Cross type	Cross	No. crosses	Corrected ratios (mottled/mottled and r^+)		Corrected mean ratio ± S.E. of mean
$rA \times rA$	$r_{47} \times r_{61}$	2	0.075	0.118	0.097±0.022
	$r_{47} \times r_{64}$	2	0.033	0.054	0.043±0.012
	$r_{47} \times r_{59}$	2	0.067	0.041	0.054±0.012
	$r_{47} \times r_{62}$	4	0.080, 0.076, 0.079, 0.044		0.07 ±0.009
$rA \times rB$	$r_{47} \times r_{43}$	4	0.053, 0.123, 0.091, 0.131		0.10 ±0.02

These crosses include the marker tw_{41} in one or other of the input parents. An analysis of variance on the corrected ratios gives an F ratio of 1.52, which is well below the F ratio of 3.63 for the 0.05 fiducial level.

plaques could possibly have resulted from superinfection of an r^+ infected bacterium by T6r_{47} which was able to enter the bacterium and recombine with the T4r^+ particles, thus giving a yield of T4r^+ and T4r_{47} on S/6.

As for the *r* plaques, they would be expected to be transmission complexes; however, they give an upper limit estimate of the efficiency of growth of the nonrecombinant heterozygotes, since, if these could grow in K bacteria, most of them should produce *r* plaques on S/6. Since one percent of the progeny-K complexes should consist of nonrecombinant heterozygote complexes, it can be concluded that less than one percent of the nonrecombinant heterozygotes produced in this cross are able to yield progeny upon infection of K.

A similar argument may be derived from the $r_{43} \times r_{73}$ cross (Table 1b). Here, because the transmission coefficient was low, the number of *r* plaques from the progeny-K complexes has been recorded. Their frequency is again 0.01 percent of the total progeny-K complexes and therefore could represent very few of the nonrecombinant heterozygotes.

In summary, these experiments indicate that two types of heterozygotes which arise in crosses involving two *r*II markers in the same cistron, may be distinguished by their ability or inability to grow in K bacteria. Those which do grow in K are r^+ recombinant heterozygotes, similar in structure to the heterozygous progeny obtained from an $r \times r^+$ cross. These appear to be a constant fraction of the r^+ recombinants within the range of map distances employed (0.1–4.0 percent). Of the other type, the nonrecombinant heterozygotes, at least 99 percent are unable to grow in K and produce progeny. They therefore have an *r*II phenotype.

Two-factor crosses involving rII markers in different cistrons

Crosses of *r*IIA × *r*IIB give no indication of yielding two classes of mottling particles differentiable on the basis of their ability or inability to infect K bacteria with the successful production of progeny. In these crosses the frequency of mottling particles relative to r^+ found among the progeny grown on S/6 is comparable to that found in crosses of the type *r*IIA × *r*IIA and *r*IIB × *r*IIB which give

a similar frequency of r^+ recombinants (see circles in Figure 4). In the latter crosses, especially for closely linked *r* markers, most of the mottling particles were demonstrated to be nonrecombinant heterozygotes, unable to grow in K. Since no such class is detected in the crosses of the type *r*IIA × *r*IIB, it would appear that the nonrecombinant heterozygotes formed in these crosses can grow in K bacteria and therefore exhibit an *r*II$^+$ phenotype.

Since K bacteria mixedly infected with *r*IIA and *r*IIB phage produce progeny, a lower multiplicity of infection for the progeny-K complexes was used than in the crosses involving *r* markers within the same cistron. Thus more infected K cells were put on the plates than in the latter experiments. This might account for the fact that, although the ratio for the progeny-K complexes is close to that of the direct plating of the progeny, it is consistently lower. In any event, the differences are quite small, inasmuch as the ratio of these values is in all cases greater than 0.8.

These experiments then, indicate that nonrecombinant heterozygotes from crosses involving *r*II markers in different cistrons may grow in K bacteria, exhibiting an *r*II$^+$ phenotype.

The detection of nonrecombinant heterozygotes

In contrast to the r^+ recombinant heterozygotes, it is probable that a considerable fraction of the nonrecombinant heterozygotes from $r \times r$ crosses are not detected as mottled plaques on S/6. From the experiments of DOERMANN and KATAJA (1957), it is to be expected that the absolute frequency of nonrecombinant heterozygotes among the progeny of an $r \times r$ cross should approach one percent as the distance between the markers is reduced. However, as can be derived from Figure 4, the absolute frequency of detected nonrecombinant heterozygotes remains less than this (less than 0.2 percent in the case of the $r_{47} \times r_{61}$ cross, and therefore about 20 percent of the expected number of nonrecombinant heterozygotes). It may be concluded that only a fraction of the actual nonrecombinant heterozygotes are detected as mottled plaques.

But in contrast to the estimated 20 percent of the heterozygotes which produce mottled plaques on S/6, less than one percent of B bacteria mixedly infected with these two *r* markers exhibit any detectable mottling. This is presumably due to the fact that, although many of these mixedly infected bacteria produce r^+ recombinants, too few are produced in each bacterium to give detectable mottling in the plaque. It therefore seems plausible that the formation of recombinants from heterozygotes is a more efficient process than from mixed infections. Additional experimental evidence to support this idea has been presented (EDGAR 1956), and a more detailed account will be published in a future paper.

These considerations do not influence the conclusion presented regarding the *r*II phenotype of nonrecombinant heterozygotes from crosses of two *r*'s in the same cistron, since, as was shown, it was possible to confirm this conclusion independently of scoring mottled plaques.

Such experiments were not performed to show that nonmottling nonrecom-

binant heterozygotes from crosses of two *r*'s in different cistrons have an *r*II$^+$ phenotype. However it would seem unwarranted, in this case only, and not the case above, to suggest that nonrecombinant heterozygotes are of two sorts with respect to phenotypic action.

DISCUSSION

The experiments described here indicate that phage heterozygous for a single pair of *r*II and *r*II$^+$ alleles are able to grow in K bacteria with very nearly the efficiency of *r*II$^+$ particles. This is also true of phage heterozygous for two *r*II markers in the *trans* position, provided the markers are in different cistrons. But if the markers are in the same cistron, the phenotype of the double heterozygotes is similar to that of *r*II mutants, in that they are unable to produce progeny upon infection of K. These findings parallel the observations of BENZER (1955) regarding K bacteria mixedly infected with phage of different genotypes corresponding to the heterozygote types above. It thus appears that no phenotypic properties have been found which are attributable to heterozygotes as distinct from the combination of parental types from which they arose.

Without knowledge of the nature of the block to phage production in *r*II-K complexes, no decision can be reached at this time regarding which of the models for the structure of heterozygotes presented in Figure 1 is correct. However, that an unaltered and intact *r*IIA$^+$ and *r*IIB$^+$ cistron must be present in a phage-infected K bacterium for the successful production of progeny is suggested by the experiments of BENZER (1955) and of KRIEG (1957). This is most easily interpreted as indicating that a cistron functions as a unit, perhaps as a template, in the formation of the primary gene product. If the structure of the heterozygote is as depicted in Figure 1a, and must function as such in a K bacterium which it infects, it would be expected that often heterozygotes of this type would have an *r*II phenotype, since, in some cases, one strand will have an incomplete cistron while the other strand will be mutant (*r*).

If some multiplication of vegetative phage may proceed in K complexes before the expression of an *r*II$^+$ phenotype is required, both structural models of heterozygotes could predict the observed results, since the replication of the segregants of the heterozygote would presumably produce a complex similar to that formed from mixed infections with the corresponding genotypes. However, a model in which the "partial replicas" (Figure 1a) multiply as such would require the assumption that, if they are unable to supplement each other structurally during self replication, then these partial replicas may supplement each other in the formation of gene product. Otherwise the *r*II $\times$ *r*II$^+$ and the *r*IIA $\times$ *r*IIB crosses would yield a detectable number of heterozygotes which would be incapable of forming *r*II$^+$ gene product.

If, on the other hand, the *r*II$^+$ phenotype must be expressed before any multiplication of vegetative phage occurs, the observations reported in this paper would seem to require that *both* strands of the heterozygote independently initiate the production of gene product. But the observation that nonrecombinant heter-

ozygotes with two *r*II markers in one cistron are unable to grow in K would suggest that no "recombinant" products of the gene are formed.

The partial replica model for the structure of the heterozygote (Figure 1a) would then require the assumption that gene product may be formed by switching templates, but at the ends of an overlap region only. These assumptions, necessary for the partial-replica model under the conditions specified, would make the "diploid" model (Figure 1b) for the structure of the heterozygote the more appealing one.

Although some preliminary evidence (KRIEG 1957) may be interpreted to suggest that no phage multiplication occurs in *r*II-K complexes, it is suggestive only, and no final conclusions can be drawn from the above arguments until more decisive evidence is at hand.

The studies reported in this paper show that, from crosses of two *r*II markers, recombinant particles which are measured on K will include certain classes of heterozygotes as well as r^+ recombinants. In crosses involving markers within the same cistron, only r^+ recombinants and r^+ recombinant heterozygotes will produce plaques on K bacteria. Since the r^+ recombinant heterozygotes constitute a small, and probably constant, fraction of these particles, this method of determining map distances by two-factor crosses will yield results that differ only slightly from the direct measurement of recombinants, and will very likely be proportional to it. Although the recombinant heterozygotes presumably represent unsegregated recombinants, until their role as intermediates in recombinant formation is more fully understood, the interpretation of studies in which they are not distinguished from recombinants may possibly be subject to qualification.

It should be pointed out that the high negative interference phenomenon described for recombination in the *r*II region (CHASE and DOERMANN 1958) is not attributable to the scoring of heterozygotes. Less than one third of the progeny particles scored on K bacteria from the cross $r_{227}r_{320} \times r_{205}$ are r^+ recombinant heterozygotes. Since CHASE and DOERMANN found a negative interference factor greater than 15 for this cross, even the elimination of the heterozygotes from the data, if reasonable, would leave virtually all of the recombinants to be otherwise explained.

In crosses involving two *r*II markers in different cistrons, nonrecombinant as well as r^+ recombinant heterozygotes will produce plaques on K. The nonrecombinant heterozygotes from this type of cross may grow in K, and if r^+ recombinants are formed during growth, they can initiate the formation of a plaque on K. In the case of closely linked markers, the nonrecombinant heterozygotes will constitute nearly one percent of the total progeny. Since heterozygotes have a high efficiency of recombination (EDGAR 1956), these will constitute a large fraction of the plaque forming particles measured on K in such crosses. It is therefore apparent that the measurement of recombination by plating on K bacteria of progeny from crosses of two *r*II markers in different cistrons will, in many cases, give spuriously high recombination values. This phenomenon may well account for the apparent "gap" between the A and the B cistrons found by BENZER (1955).

SUMMARY

The experiments reported here indicate that all, or virtually all, phage particles heterozygous for a single pair of *r*II and *r*II$^+$ alleles have an *r*II$^+$ phenotype in K12 (λ) bacteria. On the other hand, particles heterozygous at two *r*II loci in the *trans* position have a mutant phenotype in K12 (λ), provided the two markers are in the same cistron. If the markers are in different cistrons, most, if not all double heterozygotes have an *r*II$^+$ phenotype.

Certain implications arise from these observations in respect to the analysis of recombination and the structure and phenotypic action of the phage genome. Recombination analyses which utilize the *r*II-K system may be subject to some qualification, for it has been shown that no reliable estimate of the map distance between two *r*II markers in different cistrons can be made from the measurement of plaque forming particles on K12 (λ) bacteria. Although the experiments reported here do not distinguish between the "partial replica" and "diploid" models of heterozygote structure, fewer assumptions need be applied to the latter model in order to account for the results obtained.

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HETEROTHALLIC BEHAVIOR OF A HOMOTHALLIC STRAIN IN SACCHAROMYCES YEAST

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A homothallic strain of Saccharomyces yeast is invariably diploid in its vegetative state, produces ascospores in pure culture and was believed to be neutral in its mating reaction with haploid strains of either mating type. On the other hand, a heterothallic strain is characterized by its mating type; a haploid strain of mating type *a* usually conjugates only with a haploid of the opposite mating type, α, resulting in a heterozygous diploid which, when placed in an appropriate environment, segregates haploids of both *a* and α mating types.

A hybrid between a homothallic *Saccharomyces chevalieri* and a heterothallic *Sacch. cerevisiae* was created for the first time by WINGE and ROBERTS (1949). They revealed the presence of a gene controlling homothallism which segregated independently of the mating type gene. A similar sexual behavior was reported by LEUPOLD (1950, 1956a) in *Schizosaccharomyces pombe*, in which homothallism and heterothallism were considered to be under the control of four alleles of one locus.

In this paper, the authors wish to present data obtained with a hybrid between a homothallic and a heterothallic strain of *Sacch. cerevisiae*. The homothallic strain reacted as mating type α, and the hybrid gave homothallic *aa* and $a\alpha$ diploid segregants. With these strains, some crosses were attempted to produce polyploid hybrids.

MATERIALS AND METHODS

Organisms

The homothallic strain, No. S-341, is a wild type of *Sacch. cerevisiae*, which has been purified several times through single ascospore and single cell isolation. It is diploid in its vegetative state and gives a homogeneous culture in respect to cell size, spore formation, and sugar fermentation, being capable of fermenting galactose, sucrose, and maltose, but incapable of fermenting melibiose and α-methyl glucoside. As heterothallic materials, LINDEGREN's Carbondale strains (supplied by his courtesy) were employed. No. S-262 is a haploid strain of mating type α which requires adenine and arginine for growth while No. L-8256 is a haploid of mating type *a* which requires only adenine. Both of them are pink in color and unable to ferment galactose, sucrose, and maltose. One of the heterozygous diploids, No. H-315, produced by crossing these haploid strains, was employed as the heterothallic diploid stock.

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Media

The media used in this study were the same as reported previously (SAITO and TAKAHASHI 1957); modified LINDEGREN's yeast broth was used as complete medium and the minimal medium was BURKHOLDER's (see LINDEGREN 1949). The media were solidified by the addition of 15 gm. of agar per liter. For sugar fermentation tests the complete medium was modified; e.g., glucose was replaced by two percent of appropriate sugar, and an alcoholic solution of brom-cresol-purple (BCP) was added. For the same purpose, a modified medium supplemented with eosine-methylene-blue (EMB) was employed. Sometimes, the minimal medium was modified by replacing glucose with two percent of appropriate sugar, or by supplementing in addition with small amounts of adenine and/or arginine.

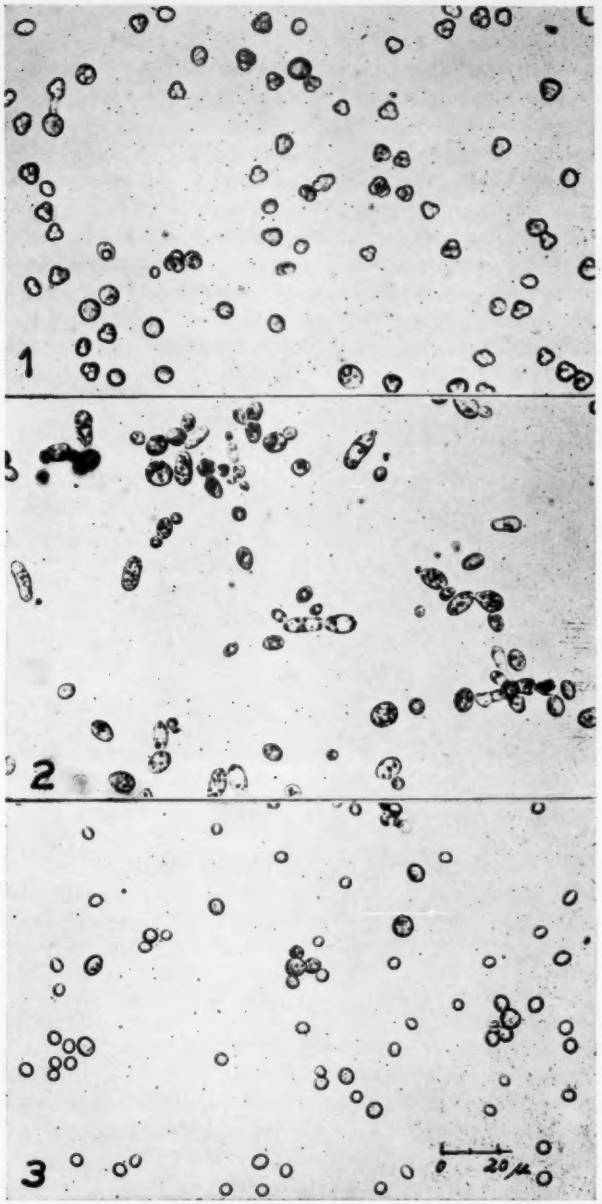
Experimental procedures

Sporulation was accelerated by inoculating large amounts of vegetative cells on Kleyn's medium (KLEYN 1954). In order to obtain ascospores with high viability, they were dissected with a micromanipulator after between 2 to 4 days incubation at 25°. Hybrids between the homothallic strain No. S-341 and the heterothallic diploid No. H-315 were produced in the following way: two ascospores, one from No. S-341 and one from No. H-315, were placed close together in a droplet of complete medium and the copulation figure was observed under a microscope. Among a number of such trials, those combinations in which new cells developed from the contact center were selected. Of course, this process was followed by single cell isolation, and the clone showing hybrid nature served for further investigation.

Nutritional requirements were determined as follows: a small number of cells was streaked on minimal, supplemented, and complete media and growth after 2 to 4 days at 25° was observed. Sugar fermentation was examined in one ml of BCP-indicator medium (HAWTHORNE 1955), and the color change after 1 to 3 days was registered. Fermentation of galactose and sucrose was further checked with Durham tubes as well as with EMB-indicator agar.

Mating type was determined by the presence or absence of copulation figures in mass culture; e.g., the unknown strain was mixed with standard haploid strains of mating type *a* or α; strains showing a positive reaction with *a* were designated α, and *vice versa*.

Homothallism was checked in two ways: by the absence of copulation figures in mass culture where the cultures arising from ascospores (spore cultures) were incubated in mixture with either heterothallic haploid, *a* or α, and by the fact that the spore cultures produced ascospores by themselves when placed on the sporulation medium. The efficiency of spore production by a homothallic culture was found, in general, to be the same order as that of the present heterothallic diploid strain. Exceptionally, some nonmating segregants from the hybrid showed no sporulation on the sporulation medium, but they were considered to be diploid for the reason that they produced plenty of cells containing granules which are characteristic of diploid cells (Figures 1–3).



FIGURES 1–3.—Mature cells on the sporulation medium after five days at 25°. Figure 1—Sporulation positive, homothallic diploid. Figure 2—Sporulation negative, homothallic diploid. Figure 3—Sporulation negative, heterothallic haploid.

In spite of the general rule that homothallic strains are incompatible with others, the strain studied here showed a mating reaction when they were tested by a special method termed "the minimal plate mating technique." This technique is grounded on the same idea as that on which POMPER and BURKHOLDER's (1949) prototroph technique is based, and goes as follows. First, homothallic diploid cells and heterothallic haploid cells of mating type *a* or *α* are streaked together on minimal or modified minimal medium. Such supplements of sugar and/or growth factor(s) were employed as needed to prevent the growth of either of the tester strains but not that of the resulting zygotes. Taking into account the fact that nonfermenters are in general slow growers, the presence of vigorously growing colonies was considered to be positive evidence for copulation (Figure 4).

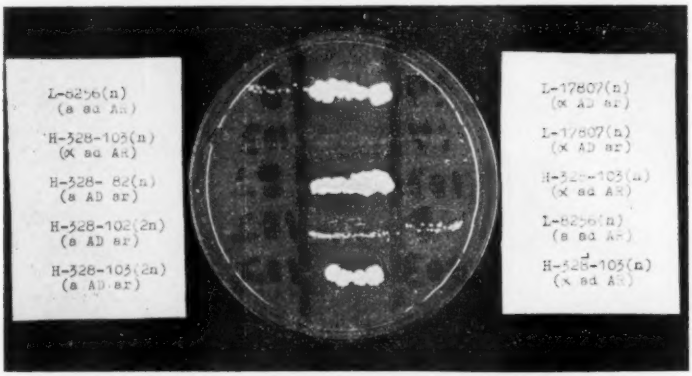


FIGURE 4.—The minimal plate mating technique. The cells of two strains were counter streaked. Growth response in the center was taken as the evidence of copulation.

If positive growth occurred in the mixed culture with the *a* type haploid, the mating type of the homothallic strain tested would be *α*. In this connection, it should be noted that no syntrophic growth has been experienced in any combinations so far tested.

Gene symbols

Genotypes are to be represented by the following symbols shown in parenthesis: mating type (*α/a*); homothallism/heterothallism (*HO/ho*); independence/dependence upon adenine (*AD/ad*), arginine (*AR/ar*); ability/inability to ferment galactose (*GA/gα*), sucrose (*SU/su*), maltose (*MA/ma*), melibiose (*ME/me*), and *α*-methyl glucoside (*MG/mg*). The adenine gene will be expressed as *AD/ad*, though, exactly speaking, the recessive allele corresponds to ad_1 in a previous paper (SAITO and TAKAHASHI 1957). Phenotypes are to be expressed by the three letter system of LINDEGREN and LINDEGREN (1956).

RESULTS

Hybridization

With reference to WINGE and ROBERTS' preceding work on hybrid formation, the cross between the homothallic strain and the heterothallic diploid was carried out by means of "the spore to spore copulation technique." Ascospores from the homothallic strain No. S-341 (white, *HO/HO*, *α/α*, *AD/AD*, *AR/AR*, *GA/GA*, *SU/SU*, *MA/MA*, *me/me*, *mg/mg*) and the heterothallic diploid strain No. H-315 (pink, *ho/ho*, *α/a*, *ad/ad*, *ar/AR*, *ga/ga*, *su/su*, *ma/ma*, *ME/ME*, *MG/MG*) were placed with a micromanipulator close together one by one in a droplet as described before. Out of 14 sets of crosses, only one which showed an apparent copulation figure was isolated. Beside this one, there were some others showing similar figures but they underwent a multidirectional development. The reason why the *α/α* genotype was given to No. S-341 is that growth was observed on melibiose or α-methyl glucoside-containing minimal medium when the strain was doubly streaked with the haploid strain, No. L-8256 of mating type *a*. Therefore the genotype of the ascospore which arose from the heterothallic strain No. H-315 and showed a copulation figure with the ascospore from the homothallic strain No. S-341 was *a* and the same as No. L-8256. This assumption agrees well with the segregation pattern to be described later.

Segregation of the hybrid H-328

The strain thus obtained (No. H-328) had a hybrid nature, and was white in appearance and capable of fermenting melibiose and α-methyl glucoside. After placing the strain on a sporulation medium, the resulting ascospores were analysed by the routine technique. About 70 percent of the ascospores dissected were viable, including eight complete asci with which ascus analysis was performed (Table 1). Among the four single spore cultures arising from each of these asci, two produced no ascospore on the sporulation medium and exhibited copulation figures when mixed with testers of either mating type. Moreover, heterozygous diploids produced between spore cultures and tester strains segregated their components in the ratio of 2:2. Whereas, the remaining two showed no apparent mating reaction, and some of them formed ascospores by themselves though the others resulted in such cells which contained granules instead of ascospores (Figs. 1–3). Then, according to the criterion given before, the former two spore cultures were considered to be heterothallic and the latter two homothallic.

The assumption that the ascospore which had taken part in the formation of the hybrid was of the genotype, pink, *ho*, *a*, *ad*, *ar*, *ga*, *su*, *ma*, *ME*, *MG*, was verified in this segregation, since *a* and *ar* were recovered in some of the offspring along with the other markers which showed regular segregation ratios. The reason why an irregular segregation was observed for the *mg* gene will be discussed later.

Minimal plate mating test

Though the homothallic segregants mentioned above showed no mating reaction with haploid testers in mass culture, it was still possible to determine their

TABLE 1

Segregation data of a hybrid produced by crossing ascospores from homothallic and heterothallic strains

Strain No.	Genotypes										Sporulation*	Minimal plate mating test succeeded/tried†
	HO/ho	α/a	AD/ad	AR/ar	GA/ga	SU/su	MA/ma	ME/me	MG/mg			
Hybrid: H-328	HO/ho	α/a	AD/ad	AR/ar	GA/ga	SU/su	MA/ma	ME	MG		+	—
Segregants:												
H-328-11	HO	α	AD	ar	ga	su	MA	me	mg	(2n)‡	+	4/11
12	ho	a	AD	AR	ga	SU	MA	ME	mg		—	
13	ho	α	ad	ar	GA	su	ma	ME	mg		—	
14	HO	a	ad	AR	GA	SU	ma	me	mg	(2n)	?	2/8
21	ho	a	ad	AR	GA	SU	MA	me	mg		—	
22	ho	α	AD	ar	ga	su	MA	ME	mg		—	
23	HO	a	AD	AR	ga	su	ma	me	mg	(2n)	?	5/7
24	HO	α	ad	ar	GA	SU	ma	ME	MG	(2n)	+	1/6
31	HO	α	AD	AR	GA	SU	MA	ME	mg	(2n)	?	2/5
32	ho	a	ad	AR	ga	su	MA	ME	mg		—	
33	ho	a	ad	ar	GA	su	ma	me	MG		—	
34	HO	α	AD	ar	ga	SU	ma	me	MG	(2n)	+	2/6
61	ho	a	ad	AR	GA	su	ma	me	MG		—	
62	HO	a	AD	AR	ga	SU	ma	me	mg	(2n)	?	3/4
63	ho	α	AD	ar	GA	su	MA	ME	mg		—	
64	HO	α	ad	ar	ga	SU	MA	ME	MG	(2n)	+	2/3
71	HO	α	AD	AR	ga	su	MA	ME	mg	(2n)	?	3/3
72	ho	a	ad	AR	GA	su	ma	me	MG		—	
73	ho	a	ad	ar	GA	SU	MA	me	MG		—	
74	HO	α	AD	ar	ga	SU	ma	ME	mg	(2n)	+	4/8
81	HO	α	ad	ar	GA	SU	MA	me	MG	(2n)	+	1/2
82	ho	a	AD	ar	GA	SU	ma	ME	mg		—	
83	HO	a	AD	AR	ga	su	MA	ME	mg	(2n)	+	1/3
84	ho	α	ad	AR	ga	su	ma	me	mg		—	
101	ho	α	AD	ar	ga	SU	ma	me	mg		—	
102	HO	a	AD	ar	ga	su	MA	ME	MG	(2n)	+	1/4
103	ho	α	ad	AR	GA	SU	MA	me	mg		—	
104	HO	a	ad	AR	GA	su	ma	ME	mg	(2n)	+	4/7
111	ho	α	AD	ar	ga	su	ma	me	mg		—	
112	HO	a	ad	ar	GA	SU	MA	ME	mg	(2n)	+	1/2
113	ho	α	AD	AR	ga	su	ma	ME	MG		—	
114	HO	a	ad	AR	GA	SU	MA	me	mg	(2n)	?	2/4

* Sporulation negative strains which form granules are indicated by interrogation marks.
† Heterothallic haploid segregants give successful results in every case.
‡ In the case of homothallic strains, the genotypes were represented by one set of symbols.

concealed mating types by means of the minimal plate mating method. As shown in Table 1, some of them required adenine and/or arginine. They were streaked together on minimal medium with haploid strains of the two mating types and with opposite requirement. The appearance of growing colonies between the tested strain and a strain with mating type *a* was considered to be evidence that the tested strain was of mating type α. As the tested strains were self-fertile and considered to be diploid, the genotype was recorded as $\alpha\alpha$. The situation might be similar for other markers and they were described as diploid in Table 1. In such cases where homothallic segregants showed no nutritional requirement, sugar fermentation characters were adopted; that is, by the use of sugar replaced minimal media, the mating types of homothallic strains were determined (Fig. 4).

This kind of experiment was carried out several times with every one of homothallic segregants. As shown in Table 1, the mating tests were sometimes successful and sometimes not, but no homothallic strain capable of mating with both *a* and α types was found. Thus it was proved that the segregation was regular even for the mating type gene.

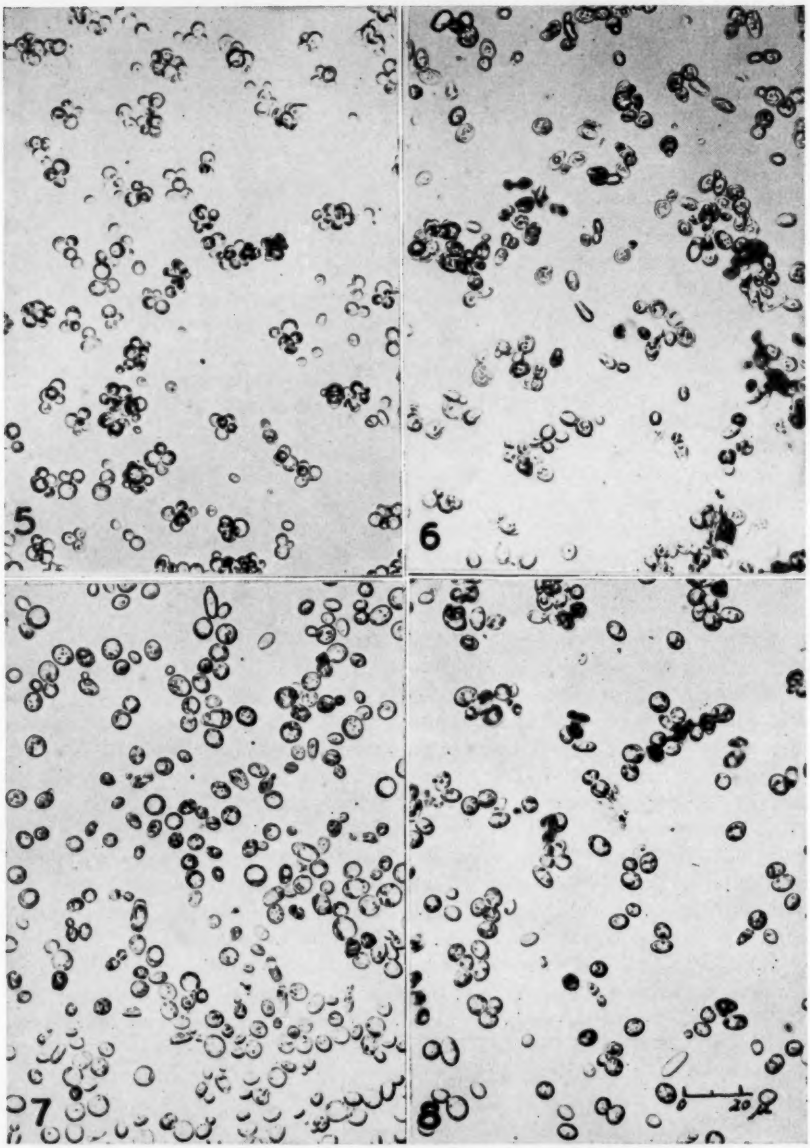
Polyploidy in the zygotes

The zygote cells secured from colonies growing on minimal plates must be triploid if copulation did occur between homothallic diploid and tester haploid cells. The strains started from these possible zygotes had larger cell sizes than any of the parents as shown in Figures 5 to 7, and showed phenotypic characters of hybrids between parents (Table 2). Therefore, it might be reasonable to make the speculation that these larger cells were triploid, although no complete ascus has yet been analysed owing to their low viability, less than ten percent of the dissected spores.

In this connection, an experiment to produce tetraploid strains through the minimal plate mating process was attempted. After streaking No. 328–23 (*a/a*, *AD/AD*, *AR/AR*, *ga/ga*, *su/su*, *me/me*) and No. 328–24 (α/α, *ad/ad*, *ar/ar*, *GA/GA*, *SU/SU*, *ME/ME*), together on galactose-minimal medium, the growing colonies were picked and the cells thus obtained, shown in Figure 8, were larger than those of the parents. One complete ascus was analysed. As seen in Table 3, the phenotypes of the four spore cultures exhibited aberrant pattern, probably owing to tetraploid segregation, though some of these segregants were unlike diploids but rather like tetraploids having the same order of cell sizes as the supposed tetraploid (Fig. 9). It might be possible to suppose that the diploid ascospores formed through meiotic division did, when they gave rise to cells, fuse together so as to result in the formation of tetraploid cultures. Further analysis of these segregants was, however, given up owing to their low viability; e.g., about 30 percent of the ascospores germinated, but two thirds of the germinating spores did not form more than 1 to 9 cells.

DISCUSSION

WINGE and ROBERTS (1949) have revealed that the homothallic yeast, *Sacch. chevalieri*, has a gene *D* which is responsible for the obligatory diploidization of



FIGURES 5–8.—Comparison of cell size among haploid, diploid, triploid and tetraploid strains. Figure 5—Haploid strain No. L-17807. Figure 6—Diploid strain No. H-328-14. Figure 7—Triploid strain No. H-419. Figure 8—Tetraploid strain No. H-392.

single spore cultures, while a heterothallic strain of *Sacch. cerevisiae* strain Yeast Foam, has the allele *d*. The hybrid produced between these two gave two kinds of spores, one kind producing diploid sporulating cultures and the other producing haploid cultures. The diploid culture was reported unable to react with any of the strains equipped with mating types *a* or *α*. For this reason, their mating type was inferred from that of the remaining two haploids in the tetrad. LEUPOLD (1956a) reported a series of alleles controlling sexuality in *Schizosaccharomyces pombe*, strain *liquefaciens*. Four alleles were reported, h^+ and h^- determining mating

TABLE 2

Check of marker traits as evidence for conjugation between homothallic diploids and tester haploids or between two homothallic diploids

Genotypes of parents		Phenotypes of the resulting polyploid strains
H-324-14	*HO a ad AR GA SU ma ME* (2n)	H-419 *ADE ARG GAL SUC mal MEB*
L-17807	*ho α AD ar GA SU ma ME*	
H-328-34	*HO α AD ar ga SU ma me* (2n)	H-441 *ADE ARG GAL SUC mal MEB*
H-336-31	*ho a ad AR GA su ma ME*	
H-328-74	*HO α AD ar ga SU ma ME* (2n)	H-593 *ADE ARG GAL SUC mal MEB*
H-328-72	*ho a ad AR GA su ma me*	
H-328-42	*HO a ad AR GA su MA me* (2n)	H-607 *ADE ARG GAL suc MAL MEB*
H-328-32	*ho α AD ar ga su ma ME*	
H-328-23	*HO a AD AR ga su ma me* (2n)	H-392 *ADE ARG GAL SUC mal MEB*
H-328-24	*HO α ad ar GA SU ma ME* (2n)	

In the case of homothallic strains, the genotypes were represented by one set of symbols.

TABLE 3

Segregation pattern of the supposed tetraploid strain H-392

Parental genotypes		
H-328-23		*HO a AD AR ga su me* (2n)
H-328-24		*HO α ad ar GA SU ME* (2n)
Tetrads (phenotypes)	H-392-61	*ADE ARG GAL SUC MEB*
	62	*ade arg GAL SUC meb*
	63	*ade arg GAL SUC MEB*
	64	*ADE ARG gal SUC MEB*
Positive:Negative		2:2 2:2 3:1 4:0 3:1

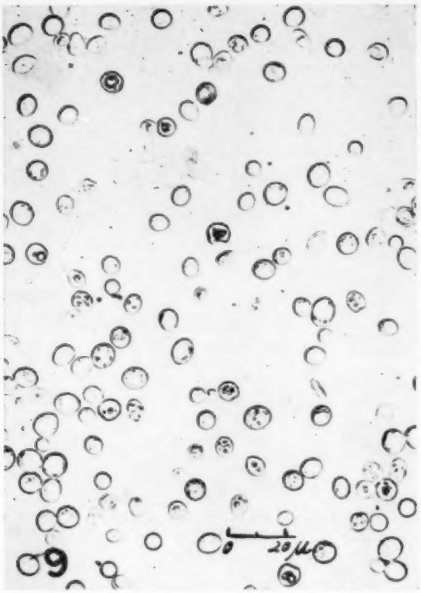


FIGURE 9.—Cells of the segregant, H-392-61, arisen from the tetraploid strain; in the same magnification as in Figure 8.

types, and H^{40} and H^{90} provoking homothallic copulation and subsequent sporulation at the rates of 40 percent and 90 percent respectively. The homothallic haploid cell was able to mate with any of H^{40}, H^{90}, h^+ and h^- but the diploidization was not obligatory.

In the present case, homothallism and heterothallism segregated 2:2, so that the situation is similar to that reported by WINGE and ROBERTS (1949). The symbols *HO* and *ho* were tentatively employed to express the homothallism-controlling-system of the present strain, since there is no assurance that *HO* is equivalent to *D*; but the dominance-recessive relationships between *HO* and *ho* is not clear. As some of the homothallic diploid segregants did not form ascospores, it might be possible to assume that the gene *HO* concerns only diploidization, but not spore formation.

It is found that the homothallic strain retains its mating potency, though not so noticeable as in the heterothallic strain. This potency was confirmed in two ways; (1) by the minimal plate mating technique and (2) by the segregation pattern of the hybrid between homothallic and heterothallic strains. This phenomenon is concerned, in some respects, to the illegitimate copulation in Carbondale strains (LINDEGREN 1949; ROMAN and SANDS 1953) and to the relative heterothallism in *Aspergillus nidulans* (HEMMONS, PONTECORVO and BUFTON 1953). But the

authors wish to discuss the matter later, after the study on the genetic background of the mating potency of the homothallic strain has been completed.

The final evidence that the larger cells produced by crossing a homothallic diploid and a heterothallic haploid and by crossing two homothallic diploid strains are really triploid and tetraploid has not yet been secured. But still the authors believe this may be so, for the following reasons: (1) growing colonies arose only from such combinations in which two mating types had been mixed, (2) the cell size of these supposed polyploid strains became larger with an increase in supposed ploidy (Fig. 5–8), (3) the phenotype of these strains agreed well with that which was expected from their genetic structures (Table 2), (4) supposed tetraploids showed aberrant segregation such as 3:1 or 4:0 with regard to some characters (Table 3). This type of segregation is expected from the segregation of tetraploid (ROMAN, PHILLIPS and SANDS 1955). In addition, the fact that the segregants produced from the supposed triploid or tetraploid strains were in general of low viability is taken as another evidence supporting this view (LEUPOLD 1956b).

As to the reason why the gene concerning the fermentation of α-methyl glucoside showed irregular segregation such as 0:4 or 1:3, the authors like to suppose that two or more genes are working together in this fermentation, in contrast to the accepted belief that the fermentation is under the control of one gene. The details are now under investigation.

In conclusion, the authors wish to stress that so-called homothallic strains of Saccharomyces yeast are not incompatible with others. They are equipped with a mating type character which, though it is not so strong as in heterothallic strains, is still detectable by special techniques such as "the minimal plate mating technique." Further, the application of this concealed potency to the study of yeast genetics will give a useful key for resolving the problems underlying homothallic and heterothallic yeast and, as well, the breeding of new types of yeast.

SUMMARY

A hybrid was produced between a homothallic and a heterothallic strain of *Saccharomyces cerevisiae* by means of "the spore to spore copulation technique." With the aid of a newly devised "minimal plate mating technique," the mating type of the homothallic strain and of homothallic segregants from the hybrid were determined, showing that mating potency does exist even in homothallic strains.

The segregation pattern of the hybrid was regular with respect to the homothallism, mating type and other characters, with the single exception of α-methyl glucoside fermentation, which was considered to be under the control of two or more genes.

From the combination of homothallic $a\alpha$ and $\alpha\alpha$ diploid strains and either of them and heterothallic α or a haploid strains, some possible tetraploid and triploid strains seem to have been produced.

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THE EFFECT OF THE LOZENGE PSEUDOALLELIC SERIES ON EYE PIGMENTATION IN DROSOPHILA MELANOGASTER. II. RED PIGMENT AND PIGMENTATION IN LOZENGE COMPOUNDS

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IN the investigation of pigmentation among the lozenge series of pseudoalleles in *Drosophila melanogaster*, the amount and distribution of red and brown pigments in the compound eyes of the lozenge mutants can be analyzed histologically by studying the genic effect on the two pigments separately. In an earlier report (CLAYTON 1957), the distribution of pigment in males of the lozenge mutants was described, as well as the distribution of brown pigment in lozenge males homozygous for the mutant brown. In continuing this study, an analysis has been made of lozenge females homozygous for vermilion (*v*) or scarlet (*st*) and eighteen compounds of females heterozygous for two different lozenge alleles but homozygous for scarlet.

OLIVER (1947) described the eye colors in ten of the lozenge alleles and the appearance of the eyes with scarlet and vermilion. GREEN (1948) analyzed quantitatively the amount of red pigment present in the eyes of seven types of lozenge females which were homozygous for a logenge mutant and for vermilion. A number of investigations have been made on the lozenge effect in compounds; these studies are discussed in an earlier paper on the structural abnormalities of the ommatidia in lozenge compounds (CLAYTON 1954a). GREEN and GREEN (1949) observed that the phenotypic effects of the lozenge mutants, when classified on the basis of the quantity of red pigment present in the eyes, were distributed at random at the three loci assigned to these pseudoalleles. In the analysis of the ommatidial abnormalities in the compounds, no correlation between the loci involved and the severity of structural defects could be found.

In the present investigation, an analysis has been made to determine, by histological methods, the distribution of red pigment in ten of the lozenge mutants and in the compounds of these mutants in order to correlate the appearance of the eyes with abnormalities in eye structure and pigment distribution.

MATERIALS AND METHODS

The histological techniques used in this study are the same as those described in the first paper of this series; heads were fixed in Carnoy's solution, sectioned at ten microns and mounted on slides unstained. Due to the solubility of the red pigment in water, some loss of pigment occurred during the dehydration process in the preparation of stained slides. The loss of pigment during the preparation

of unstained slides was slight, but those individuals in which an observable amount of red pigment was lost were not considered in the analysis.

As controls, the mutant stocks scarlet (*st*) and vermilion (*v*) of *Drosophila melanogaster* were used. Balanced isogenic stocks were used (OLIVER 1947) in which each of the following lozenge alleles was homozygous for *st* or *v*: lozenge (*lz*), lozenge 37 (lz^{37}), lozenge Bar-Stone (lz^{BS}), lozenge glossy (lz^{g}), lozenge 34k (lz^{34k}), lozenge y4 (lz^{y4}), lozenge 3 (lz^{3}), lozenge spectacle (lz^{s}), lozenge spectacle-Bishop (lz^{sB}), and lozenge 36c (lz^{36c}). The compounds examined were homozygous for the mutant scarlet and are listed in Table 1. The unstained slides of the compounds were compared with previously prepared stained slides.

TABLE 1

Comparison of eye structure and quantity of red pigment in lozenge compounds

Compound	Eye structure*	Amount of red pigment†
lz^{37}/lz	lz^{37}	Normal
lz^{37}/lz^{BS}	lz^{37}	Normal
lz^{37}/lz^{g}	lz^{37}	Intermediate
lz^{37}/lz^{3}	lz^{g}	Intermediate
lz^{37}/lz^{sB}	lz^{BS}	Normal
lz^{37}/lz^{36}	lz^{g}	Intermediate
lz/lz^{BS}	lz	lz
lz/lz^{y4}	lz^{g}	lz
lz/lz^{s}	lz	lz
lz^{BS}/lz^{g}	lz	Normal
lz^{BS}/lz^{y4}	lz^{34}	Intermediate
lz^{BS}/lz^{3}	lz^{34}	Intermediate
lz^{BS}/lz^{s}	lz^{g}	lz^{g}
lz^{g}/lz^{34}	lz^{g}	lz^{g}
lz^{g}/lz^{s}	lz^{g}	lz^{g}
lz^{g}/lz^{36}	lz^{3} or lz^{y4}	Intermediate
lz^{3}/lz^{s}	lz^{3} or lz^{y4}	lz^{3}
lz^{sB}/lz^{36}	lz^{s} or lz^{sB}	None

* From CLAYTON 1954a.
† Each allele was homozygous for the eye color mutant, *scarlet*.

RESULTS

Effect of lozenge on red pigment

The effect of the lozenge type mutant on the quantity and distribution of red pigment was studied by adding the recessive genes scarlet or vermilion to the lozenge genotype. EPHRUSSI and HEROLD (1944) reported the suppression of brown pigment by scarlet with no interference of the red pigment. The recessive vermilion also suppresses brown and leaves only red pigment. OLIVER (1947) observed that all but one of the ten lozenge alleles reduce the red pigment when *st* is present, and some of the mutants alter the red pigment distribution. He found that more red pigment is produced with *v* than with *st* and that the greatest difference between scarlet and vermilion phenotypes is among the more severe al-

leles. GREEN (1948) reported marked differences in the amount of pigment produced in nine different lozenge mutants with vermilion present. Most of the mutants used in this study were tested with both *st* and *v*. The inversion associated with lz^{sB} prevented the addition of vermilion to that genotype.

The eyes of scarlet are bright red in color, appearing in sections as light red. NOLTE (1950) noted that the postretinal pigment layer is slightly narrower in *st* than in the normal eye but that the density of the granules seems to be similar to wild type (Figure 1). The color throughout the eye is uniform, lacking the yellowish-red color found in the distal portions of the normal eye.

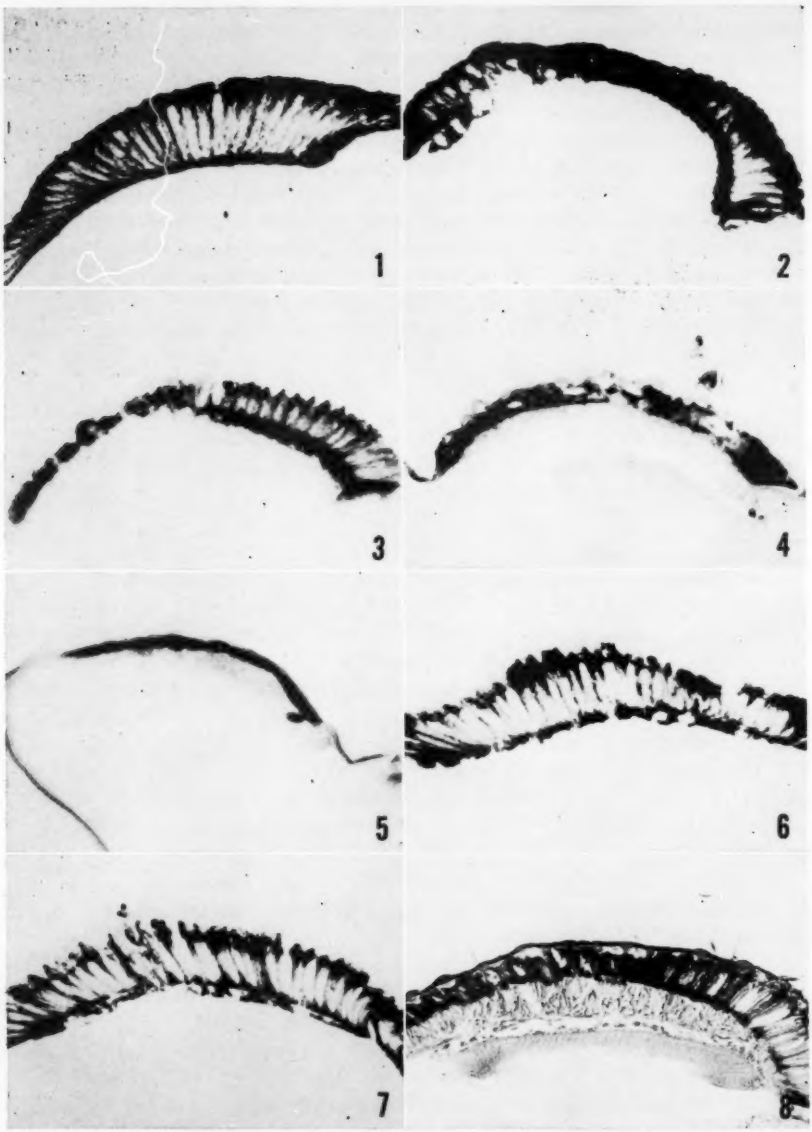
The eye of vermilion is also a bright red color. The appearance in sections is very similar to that of *st*, with a uniform light red color. JOHANNSEN (1924) described the color of *v* as being quite similar to wild type, with yellow granules sparsely distributed in the distal pigment regions and a heavy deposit of wine-red granules toward the basal regions. NOLTE (1954) described the colors of vermilion and scarlet as identical, in aggregate appearing light red. The distribution of the red granules is similar in these two mutants, and the only distinction that could be found in this study was that the over-all impression of red color seems to be slightly darker in the vermilion eye.

Lozenge 37

The least severe lozenge mutant, lz^{37}, does not appear to affect the amount of red pigment present with either *st* or *v*. The only irregularities that could be observed were associated with the small regions of structural abnormalities. The irregularities in pigment distribution are like those previously described when both red and brown pigment are present. Pigmentation in the primary cells is normal except where fusion of facets occurs, but the distortion of the pigment cells is limited to the region of the basal membrane in most areas of the eye (Fig. 2). The pigment granules are bright red in color and the amount of pigment is normal. OLIVER (1947) reported that lz^{37} was the only lozenge allele which did not reduce the amount of red pigment with either *v* or *st* in the genotype.

Lozenge

The structural abnormalities of *lz* are similar to those described in earlier papers. OLIVER described the eye color with scarlet as a light scarlet color, sometimes giving a uniform orange appearance. The color with vermilion is bright red with shiny black spots and occasional light areas. Black facet-size spots are present on the eye surface; these correspond to the "eruption" facets described previously. The color of the pigment in unstained sections is similar to that described by OLIVER. The color with *st* is orange, lighter than the bright red of the *st* mutant. There appears to be a decrease in the amount of red pigment in all regions of pigment concentration. The eye with vermilion is darker than with scarlet but the distribution is not uniform. There are occasional lighter or darker areas of pigment accompanying the structural abnormalities. The irregularities of the primary pigment cells are more marked than in lz^{37} as these cells are fre-



FIGURES 1–8.—Unstained sections of *scarlet*, lozenge pseudoalleles and lozenge compounds showing pigment patterns. Carnoy's fixative; original magnification ×200. FIGURE 1.—scarlet. FIGURE 2.—lz^{37} *st*. FIGURE 3.—lz^{BS} *st*. FIGURE 4.—lz^{9} *st*. FIGURE 5.—lz^{3} *v*. FIGURE 6.—lz^{37}/lz; *st*. FIGURE 7.—lz^{BS}/lz^{9}; *st*. FIGURE 8.—lz^{9}/lz^{34}; *st*.

quently present in a layer beneath fused facets. The secondary, basal and postretinal pigment cells are more highly distorted, with accompanying abnormalities in pigment distribution.

Lozenge Bar-Stone

The eye color of lz^{BS} with *st* or *v* is not uniform in any region of pigment deposition. The irregular distribution is closely associated with the structural abnormalities, particularly where ommatidia are absent or severely distorted. These regions differ from the normal or slightly abnormal areas by having a heavy concentration of red pigment or lacking pigment completely. With *st* the color appears orange except in severely distorted regions where a greater density results in a bright red color (Fig. 3). The effect with vermilion is similar to that with *st* except that the over-all color is a brighter red.

Glossy

The eyes of lz^{g} with either *v* or *st* are bright red except for shiny areas where fusion of facets has occurred. The appearance in sections is a bright reddish-orange except where concentration is heavier in the primary pigment cells below the fused cornea. In these regions the color is bright red. The color appears to be slightly darker with vermilion than with scarlet. A heavy concentration of red pigment occurs at the marginal rim but no granules were found accompanying the postretinal layer of retinulae (Fig. 4). The quantity of red pigment in glossy is greater than in either lz^{BS} or *lz*.

Other alleles

Both lz^{34} and lz^{y4} with scarlet have reddish-orange eyes and an irregular distribution of the pigment. In sections the rim always has a dense concentration of orange granules but the arrangement of pigment in the remainder of the eye is very irregular. The scattered distribution of the granules, except in the marginal rim, gives the impression of a yellow color. The irregular patches of pigment are concentrated beneath the fused cornea in primary pigment cells and in secondary pigment cells surrounding the postretinal layer of retinulae. With vermilion, both lz^{y4} and lz^{34} have bright reddish-orange eyes similar to the color with scarlet.

The eyes of lz^{3} *st* are pink in color as the result of scattered spots of pigment throughout the eye and a narrow pigmented rim. In sections these spots appear to be the result of small concentrations of pigment just below the cornea. Only occasional streaks of pigment could be seen below the level of the primary pigment cell layer except in the margin where a dense concentration of pigment extends from the cornea to the basal region. This allele, when vermilion is present, has much more pigment than with scarlet. The rim is heavily pigmented and the patches of red granules below the cornea are deeper red in color (Fig. 5).

The "spectacle" alleles have no observable pigment with scarlet. The sections show no pigment present in any of the regions of pigment concentration. Two of the alleles, lz^{s} and lz^{36}, were tested with vermilion and a small amount of pigment

could be observed in the marginal rim, with very small spots of pigment in other regions. The color of the pigment concentrated in the margin is a dull red, lacking the bright scarlet appearance of the less severe alleles. The small stippling of pigment below the cornea also appears to be dark red, although not as dark as the pigment of the rim.

Red pigmentation in lozenge compounds

An analysis was made of the red pigment in 18 compounds of the lozenge alleles with scarlet and the pigment irregularities were compared with the structural abnormalities described earlier (CLAYTON 1954a) and with the pigmentation in compounds described by OLIVER (1945) and OLIVER and GREEN (1947).

Compounds with lozenge 37

Six compounds of lz^{37} with other members of the lozenge series were studied. The compounds of lz^{37} with lz or lz^{BS} are similar in color to scarlet, thus normal in red pigmentation; the distal portions of the ommatidia are yellowish-orange in color and the basal regions are dark red. The structural abnormalities have been reported and the only irregularity in the red pigment distribution occurs among the disrupted basal and postretinal pigment cells in regions where abnormal postretinal elements occur. Occasionally the more distal pigment cells are disrupted by irregular retinulae penetrating the basal membrane. Such regions are usually limited to small regions of the eye (Fig. 6). OLIVER (1945) reported normal pigmentation in these compounds in a study based on the surface appearance of the eye color. The lz^{37}/lz^{9} compound is similar to lz^{37} males structurally but the red pigment appears more orange than the scarlet in structurally normal eyes. Some red pigment granules are present in irregular streaks surrounding the postretinal bundles. OLIVER (1945) reported a normal eye color in this compound. The eye structure of lz^{37}/lz^{3} is intermediate between the two alleles, resembling the glossy phenotype. The eye color of the compound as seen in sections is a dark red. The distribution of pigment is irregular due to regions of fusion and disarrangement of the ommatidial elements. In fused areas where no normal facets are present the pigment of the primary pigment cells form a dense layer beneath the flattened cornea. Some pigment cells surrounding abnormally shaped pseudocones contain only a few granules or lack pigment completely. The eye color of this compound was described by OLIVER as resembling the homozygous lz females.

The eye structure in the compound of lz^{37} with lz^{sB} is less irregular than the lz^{37}/lz^{3} compound, resembling the Bar-Stone eye. The color is a bright scarlet, very similar to that of the *st* mutant. The compounding of lz^{36} with lz^{37} results in eyes which are intermediate in both color and structure. Abnormalities in structure of the ommatidia and the deposition of red pigment granules resemble those found in lz^{9}. The outer pigment cells are yellowish-red and the basal region is a deeper red. The postretinal pigment layer beneath normal ommatidia is thicker than in *st* and a dark red pigment deposit extending distally to the primary pigment cells is present in the marginal ommatidia. Except in the marginal region,

the pigmentation is irregular in the distal portions of the secondary pigment cells and in the primary pigment cells. The color varies from yellowish-red to orange in different regions of the eye. The "erupted" facets of the cornea are frequently accompanied by a layer of primary pigment cells containing reddish-brown granules.

Other compounds

The pigmentation in two compounds with *lozenge*, lz/lz^{BS} and lz/lz^{s}, is indistinguishable from the homozygous *lz*. The lz/lz^{y4} compound produces structural abnormalities and consequently, pigment distribution like that of glossy, but the eye color is not as dark as lz^{g}, having a bright orange color rather than the darker scarlet color of the glossy mutant.

With the exception of lz^{BS}/lz^{g}, the compounds of Bar-Stone with more severe alleles are intermediate phenotypically. The structural abnormalities and red pigment distribution of the compound with glossy are like the homozygous *lz*, a condition more nearly normal than either homozygote of the compound. The color is indistinguishable from *st*. The pigment distribution in lz^{BS}/lz^{y4} is like lz^{34} and the color varies from orange to bright red with the deeper pigmentation occurring in the rim and in the region of the basement membrane. The pigment in compounds of Bar-Stone with lz^{3} and lz^{s} is bright red in the margin and bright orange or yellow in the flattened primary pigment cells below fused facets. In lz^{BS}/lz^{3} the distribution is like lz^{34} while lz^{BS}/lz^{s} is less severe, with distribution like glossy.

Three compounds with lz^{g} were analyzed; two of these, lz^{g}/lz^{34} and lz^{g}/lz^{s}, are like glossy in the amount and distribution of the red pigment. The eyes are bright red with irregularities in distribution accompanying the structural irregularities, which are also similar to glossy. Very little pigment is present in the region of the postretinal retinulae. The heterozygous combination of lz^{g} with lz^{36} is lighter in color than the other two compounds, the over-all color appearing as a bright orange with a somewhat darker rim.

The two remaining compounds studied, lz^{3}/lz^{s} and lz^{sB}/lz^{36}, possess no normal ommatidia and the cornea is flattened, with a layer of pigment cells underneath. In lz^{3}/lz^{s} a rim of orange pigment is present, appearing as a dense solid mass in primary and secondary pigment cells in that region. Small scattered masses of yellow or orange pigment granules occur beneath the cornea in the flattened primary pigment cells but their distribution is very irregular. The compound of lz^{sB}/lz^{36} contains no red pigment that can be detected in unstained material.

DISCUSSION

With this analysis of red pigmentation in the lozenge mutants and in compounds of lozenge a more complete examination of the lozenge effect on pigmentation may be made. As described in an earlier paper (CLAYTON 1957), the series of lozenge pseudoalleles fall into two groups when both red and brown pigments are present, the first group consisting of those lozenge mutants with eyes of normal

color or darker than the wild type and the second group consisting of those alleles with an extreme reduction in red pigment. When the two pigments are considered separately by eliminating either red or brown, the mutants may then be placed in linear series on the basis of their effect on either red or brown pigment, but the seriation is not the same for the two pigments.

When the different lozenge mutants are homozygous for brown the pigment distribution and the quantity of brown pigment deposited in the pigment cells make possible a seriation of decreasing pigmentation which corresponds with increasing ommatidial abnormalities of structure. The only exception to this series is that *lz*, structurally more abnormal than lz^{37}, appears to have more brown pigment present than either wild type or lz^{37}. The detailed description of brown pigmentation was presented in the previous paper by the author (1957).

The sequence of the alleles when considered on the basis of their effect on red pigment as given by OLIVER (1947) and GREEN (1948) corresponds to the seriation based on this histological study. With the exception of lz^{37}, all of the members of the lozenge series reduce the amount of red pigment produced. Both OLIVER and GREEN found that lz^{37} was normal in red pigmentation with scarlet or vermilion. Although there is a uniform reduction in the amount of red pigment in all regions of *lz* eyes, the distribution is normal except in fused regions. Green found that *lz* with vermilion had a red pigment value of only 29.2 percent relative to 100 percent value for the vermilion mutant. OLIVER reported that the eyes of *lz v* had more red pigment than with *st* and the same condition was found in this investigation. In structurally abnormal regions, however, *lz v* is more variable in the amounts of red pigment deposited than in *lz st* where the amount of red pigment even in fused areas appears to be uniform.

Both lz^{BS} and lz^{g} possess more red pigment than *lz*, a factor contributing to the darker eye color of these mutants. GREEN (1948) found the quantity of red pigment in Bar-Stone with vermilion to be 41.6 percent with lz^{g} *v* possessing 50.3 percent of the maximum. In both of these mutants the distribution is irregular and the color varies from bright orange to deep red in different regions of the eye. The eyes of lz^{34} are similar to *lz* in the amount of red pigment produced; according to GREEN's analysis the values are 29.2 percent for *lz* and 27.3 percent for lz^{34}. These two mutants, which are quite easily distinguished on the basis of eye structure, are alike in their effect on red pigment, and the darker eye color of lz^{34} may be partially explained on the basis of differences in the distribution of the pigment granules in relation to the structural abnormalities. Although the eyes of lz^{v4} appear darker than any of the previously mentioned alleles, the total amount of red pigment is considerably reduced. The localization of the pigment below the cornea would make the eye appear darker than in those mutants with elongated secondary pigment cells and definite basal and postretinal concentrations of red pigment.

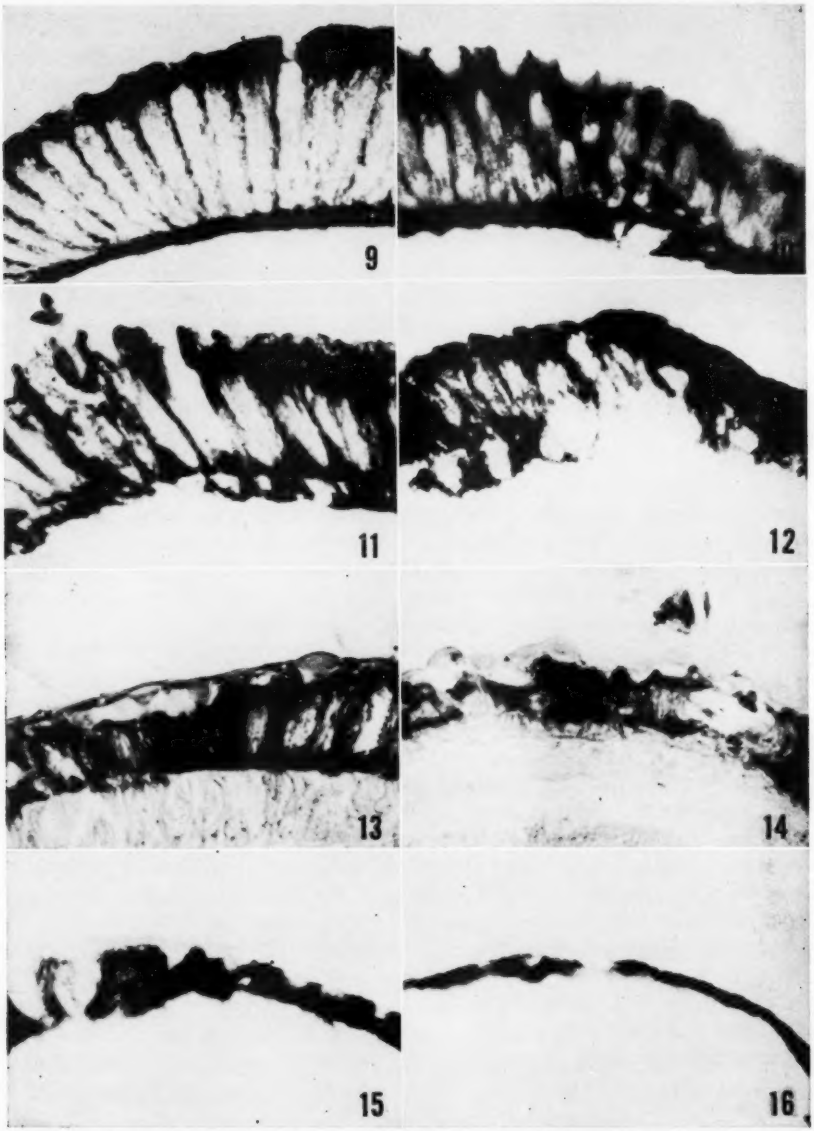
In the remaining mutants, lz^{3}, lz^{s}, lz^{sB}, and lz^{36}, the amount of red pigment in the pigment cells is greatly reduced. In this group, however, differences between the gene action with *st* and with *v* become quite apparent. With scarlet, lz^{3} has a

red marginal rim and small spots of red pigment beneath the cornea; with vermilion the pigment deposition is much greater. The rim is heavily pigmented and the remaining pigmented areas are a much deeper red. The lz^s, lz^{sB} and lz^{36} alleles have no observable pigment with *st;* however, with vermilion both lz^s and lz^{36} have dark red granules in the rim and very small widely scattered spots of dark red pigment in central regions of the eyes. GREEN (1948) found that the red pigment value for lz^3 *v* was about six percent and that red pigment could be extracted from lz^s *v* and lz^{36} *v* but the quantity was so small that it could not be measured.

The differences in eye color of the various lozenge mutants are closely related to the amount of pigment deposited in the ommatidial cells and the degree of cellular abnormality. Whether the concentration of pigment in the cells is due to differences in the number of granules present or to the amount of pigment deposited in each granule could not be determined accurately. In those individuals where counts were attempted, the number of granules did not appear to vary significantly. Since the changes in eye color occur primarily in those mutants with rather severe structural irregularities of the ommatidia, pigmentation and eye structure must be considered together. A comparison of photographs of unstained sections (Figs. 9 through 16) indicates the extent of the irregularities in red pigment distribution. The figures are arranged in the order of increasing distortion of the pigment pattern, from the normal condition of scarlet (Fig. 9) to those intermediate in abnormalities (Figs. 10 through 13) and those individuals in which the ommatidia are highly distorted or absent (Figs. 14, 15, 16).

In lz^{37}, where distribution of pigment is normal, the disruption of the pigment cells occurs only in the region of the basal and postretinal pigment concentrations and does not affect the eye color. This type of disturbance may be seen in Figure 11. Disruption of distal pigment cells is usually limited to small regions (Fig. 12). In the remaining mutants, however, irregular distribution of the pigment occurs in primary and secondary pigment cells as well as the more proximal pigmented areas, thus affecting the over-all eye color.

Fusion of facets and a changed orientation of the primary pigment cells around abnormal pseudocones alter the relationship between the eye surface and the pigment of these cells. This probably accounts for the variations in surface eye color when the amount of pigment remains almost normal. Severe corneal abnormalities accompany an extreme disruption of the primary pigment cells and the distal portions of the secondary pigment cells. In those regions where the cornea has large depressions and pseudocones are absent, the pigment may appear as a solid layer beneath the depression, or the pigment cells may be completely absent beneath the depressed area, forming masses of heavily pigmented cells around the unpigmented region (Figs. 13, 14). This results in a colorless spot surrounded by a heavy deposit of pigment. CASTEEL (1929) described the "pink" appearance of ommatidia from surface view, but he found that these ommatidia were without pigment and owed their color to light reflected from pigment of adjacent ommatidia. OLIVER (1947) suggested that the orange appearance of the eyes of lz^{34}



FIGURES 9–16.—Unstained sections of *scarlet*, lozenge pseudoalleles and lozenge compounds selected to illustrate varying degrees of irregularities in pigment distribution. Carnoy's fixative; original magnification ×475. FIGURE 9.—*st*. FIGURE 10.—lz^{37}/lz; *st*. FIGURE 11.—lz^{BS}/lz^{9}; *st*. FIGURE 12.—lz^{37} *st*. FIGURE 13.—lz^{9}/lz^{34}; *st*. FIGURE 14.—lz^{9} *st*. FIGURE 15.—lz^{9}/lz^{36}; *st*. FIGURE 16.—lz^{3} *v*.

with scarlet is due to reflection of light through clear areas around which the red pigment is aggregated.

From the results of this study, it appears that this abnormal arrangement of the primary pigment cells together with the amounts of red and brown pigments deposited in the granules explains the appearance of irregular patches and streaks of color characteristic of the more severe lozenge mutants. In addition to depressions in the cornea, small facet-sized "erupted" facets occur in some of the mutants. These are the result of the elongation during pupal development of displaced cells above the pseudocone region (CLAYTON 1954b). Pigment distribution below such facets is similar to that beneath depressed regions; primary pigment cells may form a solid layer or may be aggregated around the eruptions.

The distortion of the secondary pigment cells which accompanies abnormalities of the retinulae also causes disruption of the normal pigment pattern. However, the most severely affected region is that of the basement membrane and postretina. The disruption of the basement membrane by abnormal retinulae in the postretina causes irregularities in the pigment distribution. The postretinal pigment regularly forms streaks of color about the postretinal retinulae in the least severe alleles, but with increasing severity of the structural abnormalities this pigment is sparse or absent, as may be seen by comparing Figures 12 and 13. Retinulae which penetrate the basement membrane produce small regions lacking pigment which are surrounded by heavy concentrations of basal and postretinal pigment.

The lz^3 mutant and the spectacle alleles, which show the severest structural abnormalities, also have the greatest irregularities in pigmentation. The heavy pigment deposit of the rim is present in elongated pigment cells at the margin of the eye, and the remainder of the pigment is deposited just below the cornea (Fig. 16). As observed in the development of lz^s (CLAYTON 1954b), many pigment cells outside the marginal layer appear to undergo degeneration during late pupal development. The small scattered spots of pigment seen in the adult eyes may be the result of this degeneration of pigment cells.

In the comparison of the effects of the mutants with *st*, *v* or *bw*, no differences in the severity of structural abnormalities were observed and differences in the distribution of pigment were not noticeable except in the postretinal concentration. With brown, the streaks of pigment accompany postretinal retinulae less frequently than with either scarlet or vermilion and, in several alleles, no pigment could be seen around these cells when only brown pigment was present.

The phenotypic effects on ommatidial structure and red pigment quantity of lozenge compounds are compared in Table 1. In the majority of the compounds the amount of red pigment corresponds to the severity of ommatidial abnormalities. However, in several compounds the dominance relationships are altered. In lz^{37}/lz^9 the structure is like lz^{37} but the eye color is intermediate; lz^{37}/lz^{4B} is intermediate in eye structure but resembles lz^{37} in the quantity of red pigment present. Although the structure of lz/lz^{w4} resembles the glossy allele, the eyes lack the heavy deposit of red pigment characteristic of this mutant and it more closely resembles *lz*. The amount of red pigment in lz^{BS}/lz^9 cannot be distinguished from

scarlet while the structural characteristics are like *lz*. No correlation in interaction between the alleles and their loci (GREEN unpublished, cited in CLAYTON 1954a) could be found on the basis of the quantity or distribution of the eye pigments. GREEN and GREEN (1949) found that the mutants, when classified quantitatively with respect to the amount of red eye pigment formed, were distributed at random to the three lozenge loci; the histological study showed a similar random distribution.

OLIVER (1947) suggested that the eye color of the lozenge alleles was the result of both the quantities of red and brown pigments produced and the distribution of these pigments. The results of the present series of investigations indicate that both of these factors are involved in the final expression of eye color. The effect of the alleles on the production of red and brown pigment appears to be at least partially independent of the ommatidial abnormalities and the resulting irregularities in pigment distribution. Although the abnormalities in the arrangement and structure of the pigment cells, together with degeneration of cells in the severe alleles, can account for variations in the distribution of pigment and some quantitative changes, other factors seem to be involved. For example, lz^s and lz^{ps} cannot be distinguished on the basis of structural abnormalities, ommatidial arrangement, or pigment distribution; yet the eye colors are quite different as a result of variations in the amount of pigment present. Both lz^s and lz^{sB} have the same ommatidial structure and eye color but act quite differently in compounds with other lozenge alleles.

An investigation on the pleiotropic effects of specific mutants in mice, carried out by GRUNEBERG (1938, 1943), revealed that the multiple effects could be traced to a single biochemical change wrought by the mutant gene early in development. In the lozenge series of pseudoalleles, however, no single factor has been discovered which will explain all of the phenotypic effects of these genes. The effect of lozenge on the color of the compound eye can be explained in part on the primary effect on eye structure. The irregular cell differentiation during development of the eye results in abnormal ommatidia and secondarily in irregular pigment distribution. ANDERSON (1945) described a similar relationship in which reduced fertility in lozenge was a secondary effect of the abnormal development of the female genitalia. Although lack of normal cell differentiation may account for several of the pleiotropic effects of the lozenge pseudoalleles, some independence in the gene expression still seems to exist. Variations in the interaction of phenotypically similar lozenge mutants in compounds, semi-dominance of fertility as compared to recessiveness of other effects, as well as differences in the quantities of red and brown pigments indicate that a complex system of gene action is involved in the final expression of the lozenge phenotype.

SUMMARY

The quantity and distribution of pigment in ten lozenge pseudoalleles homozygous for scarlet or vermilion are described and the red pigmentation in eighteen compounds from lozenge alleles homozygous for scarlet are analyzed.

The differences in eye color are the result of variations in the amount of red pigment produced and the distribution of the pigment. The distribution appears to be directly related to the abnormalities in structure and arrangement of the ommatidial cells.

Abnormal cell differentiation during pupal development and degeneration of some abnormal pigment cells may account for several of the lozenge effects but does not satisfactorily explain differences in the amount of pigment deposited in the different alleles or variations in the expression of phenotypically similar mutants when in compounds.

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MATERNALLY INHERITED "SEX-RATIO" CONDITIONS IN DROSOPHILA WILLISTONI AND DROSOPHILA PAULISTORUM

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MAGNI (1952, 1954) and CAVALCANTI and FALCÃO (1954) have independently discovered strains of *Drosophila bifasciata* and of *Drosophila prosaltans*, respectively, which produce progenies consisting mostly or only of females. These abnormalities are transmitted exclusively from mothers to their daughters. Females from the "sex-ratio" strains produce unisexual, or almost unisexual, progenies when crossed to males from a variety of strains giving normal proportions of the two sexes; at the same time the occasional sons of "sex-ratio" mothers do not differ in their breeding behavior from normal males. The abnormal "sex-ratio" condition is thus transmitted through the female line only, leading to the conclusion that the factor causing this condition is inherited in the cytoplasm. The "sex-ratio" conditions in *D. bifasciata* and *D. prosaltans* are, therefore, completely different from those described and studied much earlier by GERSHENSON (1928), STURTEVANT and DOBZHANSKY (1936), DOBZHANSKY (1939) and others in *D. pseudoobscura*, *D. persimilis*, *D. azteca* and other species. In these latter, the abnormality is transmitted through the X chromosome, in such a way that a "sex-ratio" male produces mostly or only daughters regardless of the genotype of its mates.

The purpose of the present article is to report the existence of maternally inherited "sex-ratio" conditions in two new species, namely in *D. willistoni* and *D. paulistorum*. Interestingly enough, these new cases differ in not unimportant details of their behavior, both from those in *D. bifasciata and D. prosaltans*, and from one another. The present paper is concerned with the inheritance of these "sex-ratio" cases in various crosses; the embryological aspects of the situation will be described in a paper jointly with PROFESSOR D. F. POULSON of Yale University. The transmission of the causative agent by injection of the oöplasm of the infected eggs into normal females has been reported in a preliminary communication by MALOGOLOWKIN and POULSON (1957), and it is hoped that a more detailed report will follow.

Origin of the material

Owing to the courtesy of PROFESSORS WILSON STONE and MARSHALL WHEELER of the University of Texas, samples of the population of *willistoni*-like species of Drosophila collected by Drs. W. B. HEED and M. WASSERMAN in the West Indies and in Colombia were sent for study to the laboratory of PROFESSOR TH. DOB-

ZHANSKY in New York. The females collected in their natural habitats were placed, after they had been received at Columbia University, in individual culture bottles, and allowed to produce offspring (females collected in nature are usually inseminated). Among numerous females of *D. willistoni* collected at Bath, Jamaica, on February 21–25, 1956, there was one which, when examined by MR. B. SPASSKY, proved to have produced several dozen daughters and no sons (no exact count has been made). MR. SPASSKY crossed some of these daughters to males from the laboratory strain containing the sex-linked mutants eosin (w^e), singed (*sn*), yellow (*y*), Incomplete (*Inc*), and roughoid (*ru*). The next generation consisted of numerous females and some few males. MR. SPASSKY continued the backcrossing of the females from the "sex-ratio" stock to w^e *sn y Inc ru* males for four more generations until November, 1956, when he most generously passed it to the present writer for study. The strain consisted exclusively of females, and from then on it has been maintained by crossing its females to w^e *sn y Inc ru* males from the mutant stock (which, of course, showed a normal sex ratio). The "sex-ratio" strain soon became, and continued to be, homozygous for the mutants indicated above.

A sample of *willistoni*-like flies was collected on September 6, 1956, on the southern exposure of Sierra Nevada de Santa Marta, in Colombia. On October 12, 1956, MR. B. SPASSKY found that one of the females, which belonged to the species of *D. paulistorum* (a close relative of *D. willistoni*), had produced a progeny of 26 females and no males. MR. SPASSKY very generously handed this culture to the present writer for study. A part of the females were crossed to males from another strain of *D. paulistorum* derived from the same locality. A strain of the "sex-ratio" was established from these crosses.

The "sex-ratio" strains of both species are maintained in the laboratory at room temperatures by outcrossing in every generation some of the "sex-ratio" females to w^e *sn y Inc ru* males (in the case of *D. willistoni*), or to males from the strain Santa Marta No. 6 (in the case of *D. paulistorum*).

RESULTS

Inheritance of "sex-ratio" in D. willistoni *in crosses to different strains of this species*

Females from the "sex-ratio" strain have been crossed and backcrossed to males of several other strains from the collection maintained at Columbia University. Usually groups of 4–5 females from the "sex-ratio" strains were mated to equal numbers of males of the kinds chosen for this purpose. Complete counts of the progenies were made. Most of them are summarized in Tables 1 and 2.

In the first place, the "sex-ratio" strain was outcrossed to mutant strains which contained genetic markers known to be located in each of the three pairs of the chromosomes which the species possesses (SPASSKY and DOBZHANSKY 1950). One of the purposes of these crosses was to replace the chromosomes of the original Jamaican "sex-ratio" strain by chromosomes of other origins. The results are shown in Table 1. Later on, similar crosses and backcrosses were made to some

TABLE 1

Numbers of flies counted and percent of males in crosses and backcrosses of "sex-ratio" females to males from certain mutant strains

Generation	Flies counted	Percent males	Generation	Flies counted	Percent males	Generation	Flies counted	Percent males
w^e sn y Inc ru males			*w^e sn y Inc ru* males			*abb bw* males		
5	500	0	19	346	0	2B	82	0
6	793	0	20	286	0	3B	281	30.6
7	617	0	21	146	0	pink males		
8	606	0	22	367	0	1A	2667	0
9	348	2.8	23	169	0	2A	2438	0
10	285	0	24	166	0	3A	439	0
11	692	0	*abb bw* males			..	....	..
12	742	0	1A	1699	0	1B	832	0
13	206	0	2A	1243	1.8	2B	428	0
14	338	0	3A	397	0.1	3B	140	0.7
15	90	0	4A	474	15.4	4B	265	0.3
16	285	0	5A	1249	42.6	ebony males		
17	102	0	..	....	..	1A	216	0
18	56	0	1B	415	0	2A	1341	0
						3A	479	0
						4A	217	0

TABLE 2

Numbers of flies counted and percent of males in crosses and backcrosses of "sex-ratio" females to males from certain wild strains

Generation	Flies counted	Percent males	Generation	Flies counted	Percent males	Generation	Flies counted	Percent males
Barbados-5 males			Jamaica-9 males			Recife-3 males		
1A	381	0	1B	421	0	1A	1333	0.2
2A	43	0	2B	1713	0	2A	1067	3.3
3A	28	0	3B	930	0	3A	563	20.0
1B	207	0	4B	416	13.5	4A	108	44.4
2B	4	0	St. Kitts males			5A	490	51.4
3B	223	0	1A	477	0	1B	499	0
Costa Rica-2 males			2A	594	0	2B	1385	11.7
1A	781	0	3A	489	2.2	3B	838	20.0
2A	403	0	4A	974	1.0	Recife-6 males		
3A	201	0.5	1B	332	0	1A	196	0
4A	967	0	2B	1751	0.05	2A	992	3.3
1B	577	0	3B	1293	0	3A	90	22.2
2B	1036	0	4B	276	0	1B	318	0
3B	1045	0	St. Lucia males			2B	1206	17.5
4B	342	0	1A	520	0	3B	758	36.5
Jamaica-9 males			2A	387	0	Recife-8 males		
1A	164	0	3A	267	10.5	1A	662	0
2A	690	0	4A	1002	1.9	2A	927	0
3A	517	0.2	1B	227	0.4	3A	432	9.7
4A	1573	0.06	2B	740	6.8	4A	1017	0.1
			3B	105	4.7			

strains of diverse geographic origins which contained no obvious mutant genes. These data are summarized in Table 2.

Backcrosses to *w^e sn y Inc ru* males were made and counted for 24 generations (in the first four of them they were made but not counted by MR. B. SPASSKY). As shown in Table 1, the entire progenies consisted exclusively of females, except in the ninth generation in which one of the two cultures made produced 148 females and 10 males. In the tenth generation, when all the flies in the cultures showed all the sex-linked mutant genes indicated above, some females were crossed to males containing the second chromosome mutants Star (*S*), Hooked (*Hk*), abbreviated (*abb*), and brown (*bw*). This cross, denoted in Table 1 as generation 1A, produced, in three cultures, 1,699 females and no males. Star Hooked females were next selected and crossed to *abb bw* males; the progeny, generation 2A in Table 1, consisted of 1,243 flies among which 23 were males. Three further backcrosses, 3A to 5A in Table 1, resulted in a breakdown of the "sex-ratio" condition. In the generation 5A, three cultures produced each only slight excesses of females over males. To insure that this breakdown of the "sex-ratio" condition was not accidental, the experiment was repeated by making new crosses of females from the *w^e sn y Inc ru* "sex-ratio" stock to *S Hk abb bw* males, and backcrosses to *abb bw* males. The results are summarized in Table 1 under the designation of generation 1B to 3B. The second backcross again resulted in production of 30.6 percent of males in the cultures.

In the 11th generation of the *w^e sn y Inc ru* "sex-ratio" lines, some females were crossed to males from the stock containing the mutants Delta and pink (third chromosome). The F_1 females showing Delta were crossed to pink males for two generations (1A to 3A in Table 1). A repetition of the backcrosses for four generations (1B to 4B) was made later. Except for single males in the 3B and 4B generations, the progenies consisted exclusively of females. The "sex-ratio" strain was also crossed and backcrossed for four generations to the mutant ebony (third chromosome). The results were unisexual female progenies (Table 1).

Table 2 summarizes the results of crossing and backcrossing females from the *w^e sn y Inc ru* "sex-ratio" stock to males from 8 wild stocks the progenitors of which were collected on the islands of Barbados, St. Kitts, St. Lucia, Jamaica, and also in Costa Rica and at Recife, Brazil. Each set of experiments was repeated twice, except for that with the strain Recife-8. The two series are reported in Table 2 with letters A and B respectively after the number of the generation of the cross.

The results fall into three fairly noticeably distinct classes. To the first of these classes belong the crosses to Barbados and Costa Rica. In these crosses the unisexual progenies are maintained intact with rare exceptions (three males in the generation 3A from the backcross to Costa Rica). The second class are the crosses to the strains from Jamaica, St. Kitts, St. Lucia, and Recife-8. Here the progenies of the first cross and backcross to the respective geographic lines usually consist of females only, while further backcrosses produce some males, but still nothing approaching the normal 1:1 sex ratio. Finally, the third class consists of the

strains Recife-3 and Recife-6. Crossing "sex-ratio" females to males from these strains results usually in unisexual progenies in the F_1 generation, the first backcross gives some males in every culture, while from the second backcross on, the normal sex ratio is approached in most cultures.

Nontransmission of the "sex-ratio" condition through the males

Whenever the cultures of the "sex-ratio" lines first produced a few males among numerous female sibs, attempts were made to cross these males to their sisters or to unrelated strains giving normal sex ratios, to see whether such males may transmit to their offspring a tendency to produce higher proportions of males. Outcrosses to unrelated females gave completely negative results; in seven series of such outcrosses a total of 1,329 females and 1,331 males were produced. This is obviously a normal proportion of the sexes.

The outcrosses of the males sporadically occurring in "sex-ratio" cultures to their sisters gave results summarized in Table 3. Examination of these results shows no consistent influence of the male on the sex ratio in its progeny. Thus, the ninth generation of the *w^e sn y Inc ru* "sex-ratio" line was the only one which happened to give some males (see above). These males crossed to their sisters produced unisexual progenies (a total of 285 females and no males, Table 3). On the contrary, males from the outcrosses to *abb bw*, St. Lucia, and Recife strains gave, when outcrossed to their sisters, progenies which did include males. However, comparison of the data in Table 3 with those in Table 2 shows that these results do not differ from those obtained when other sisters of the same males were mated to unrelated males from certain strains.

Lacks of temperature effect

MAGNI (1954) discovered that the "sex-ratio" condition in *D. bifasciata*, which is inherited in a manner similar to that described here for *D. willistoni*, can be "cured" by exposure of the flies to temperatures higher than optimal for the

TABLE 3

Progenies of females from "sex-ratio" strains which have produced some males, outcrossed to their brothers

Strain males	Generation	Flies counted	Males percent
w^e sy y Inc ru	9th (see Table 1)	285	0
abb bw	2A (see Table 2)	230	43.5
abb bw	3A (see Table 2)	289	2.8
abb bw	4A (see Table 2)	635	47.2
pink	F_1 (see Table 1)	60	0
St. Kitts	3A (see Table 2)	59	0
St. Lucia	3A (see Table 2)	284	37.1
Recife-3	2A (see Table 2)	195	31.3
Recife-3	2B (see Table 2)	425	0
Recife-6	2A (see Table 2)	375	44.8

species. In the case of *D. bifasciata*, MAGNI obtained only a few males from eggs deposited at 21° C, but normal sex ratios were approached when ovipositing females were exposed for several days to 26° C. Such thermic "cures" have also been observed for another cytoplasmically inherited character, sensitivity to carbon dioxide, in *D. melanogaster* (L'HERITIER 1951). Experiments have therefore been devised to test the effects of high temperatures on the "sex-ratio" in *D. willistoni*.

Although being a species native in the tropical zone of the Western Hemisphere, *D. willistoni* does not easily tolerate under laboratory conditions a temperature of 28° C for several generations. The experiments were therefore arranged as follows. "Sex-ratio" females from the *w^e sn y Inc ru* "sex-ratio" strain developed at room temperatures (about 22° C) were crossed to males from other strains, and the cultures were placed for five days in an incubator at 25° C. The eggs deposited in these cultures were allowed to develop at the same temperature, and the distribution of the sexes among the adult flies which hatched is reported in the upper line "Generation P, 25° C" in Table 4. The parents were next transferred to fresh culture bottles which were placed for a 5-day period in an incubator at 28° C. The progeny hatching from the eggs deposited at that temperature developed also at 28°; the results are presented in the line "Generation P, 28° C" in Table 4. Finally, the surviving parents were transferred once more to fresh cultures, returned to 25° for another 5-day period, and the progeny allowed to develop at the same temperature at which the oviposition occurred. The results are found in the lower line "Generation P, 25° C" in Table 4.

The flies developed at a given temperature were then allowed to mate and to oviposit at that same temperature for three generations. The results are reported

TABLE 4

Numbers of flies counted and percent of males in crosses and backcrosses of "sex-ratio" females to males of three groups of strains carried out at different temperatures. (Further explanation in text)

Generation	t° C	First group		Second group		Third group	
		Flies counted	Percent males	Flies counted	Percent males	Flies counted	Percent males
P	25°	1447	0.2	3598	0.3	1380	0.6
	28°	771	1.2	845	0.7	329	0
	25°	1186	0	1151	0	463	9.5
F_1	25°	3750	0	5692	1.1	3962	10.5
	28°	1402	2.8	1702	5.3	3916	8.4
F_2	25°	1631	0	2353	0.04	2789	23.4
	28°	1132	0	860	0.1	4204	10.5
	25°	....	..	....	..	2583	6.1
F_3	25°	970	0	957	5.9	....	..
	28°	400	0	440	0	608	5.0

in Table 4 in the lines marked F_1, F_2, and F_3, 25° and 28°. However, in the F_2 and the F_3, 28° lines became so weak that their loss was threatened; for this reason, the progeny from the eggs deposited at 28° was allowed to develop to maturity at 25°.

In Table 4, the crosses are divided in three groups, corresponding to the behavior of the respective strains in the experiments carried out at room temperatures and reported in Tables 1 and 2 (see above). The data in Table 4 should, hence, be compared with those in Tables 1 and 2. The first group (crosses to *w^e sn y Inc ru*, to pink, ebony, Barbados, and to Costa Rica) has produced almost pure female progenies, except for scattered males in several cultures of crosses to pink, Barbardos, and Costa Rica at 28°. The F_1 generation also produced a few males at 28°, but none appeared in F_2 or F_3.

To the second group, belong the crosses to strains from Jamaica, St. Kitts, St. Lucia, and Recife-8. In these crosses (Table 4) the progenies consisted predominantly of females except for about five percent males in the F_1 generation at 28°, and about six percent males in the F_3 generation at 25°. Finally, the third group contains the crosses to *abb bw*, Recife-3, and Recife-6. The progeny of the first outcross (P) which was produced at 25° and at 28° consisted, as usual, of females and only a scattering of males, but the outcross to *abb bw* gave 26 females and 29 males when returned to 25° from 28°. The outcross to Recife-3 gave 195 females and 15 males, while the outcross to Recife-6 produced only females. In the backcrosses males appeared in most cultures both at 25° and at 28°, and no consistent relationship with temperatures at which the flies developed was noticeable.

Reference to Tables 1 and 2 will show that the three groups of strains behaved differently also in the crosses to the "sex-ratio" stock carried out at room temperatures. The third group of strains yielded numerous males, or even normal proportions of the two sexes beginning in the second backcross. There is, hence, no consistent difference between the crosses at room temperature, at 25° and at 28°. No thermal "cure" of the "sex-ratio" condition is observed in *D. willistoni*, although the possibility is not excluded that the scattered males which occur in the "sex-ratio" stock are slightly more frequent at 28°.

Permanent loss of "sex-ratio"

At whatever temperature a strain of "sex-ratio" females crossed to males from strains of the third group (see above) gives normal proportions of sexes, the condition becomes permanent from then on. Females from such strains were outcrossed to a variety of males and their progenies showed no restoration of the capacity to produce unisexual progenies.

"Sex-ratio" in Drosophila paulistorum

The "sex-ratio" condition in *D. paulistorum*, although inherited in a manner generally similar to that in *D. willistoni*, *D. prosaltans*, and *D. bifasciata*, has certain characteristics which are not found in the other species. As shown above,

the "sex-ratio" in *D. willistoni* has a very nearly absolute character, producing almost completely unisexual progenies, unless outcrossed to strains of a certain group which evidently contain genes which disrupt the transmission of the "sex-ratio" causative agent. This is not the case in *D. paulistorum*.

Fourteen females were taken from the culture of the "sex-ratio" of *D. paulistorum* obtained from MR. B. SPASSKY and outcrossed in individual cultures to single males from the strains Santa Marta-144 and Santa Marta-185. These strains came, then, from wild progenitors collected in the same natural locality as the "sex-ratio" strain itself. Table 5 shows the results. Nine of the females produced progenies consisting of females only, while five females gave approximately equal numbers of females and males.

TABLE 5

Progenies of single females from the original "sex-ratio" cultures of Drosophila paulistorum *crossed to males from a normal strain*

Culture	Total number flies	Percent males	Culture	Total number flies	Percent males
I	67	0	VIII	134	0
II	65	0	IX	98	0
III	48	0	X	152	0
IV	104	0	XI	272	48.5
V	12	41.6	XII	132	50.0
VI	58	50.0	XIII	110	0
VII	61	49.1	XIV	9	0

The cultures "sex-ratio" I, II, III, and VI were chosen for further study (cf. Table 5). In each generation, single females were taken at random from the lines I, II, III, and VI, and were mated with single males from the strains VI and XI. As can be seen in Table 5, the strains I, II, and III originally produced strictly unisexual progenies, while VI and XI gave approximately equal numbers of females and males. The results are summarized in Table 6. The lines I and II have continued for at least 12 generations (and are still continuing at the date of this writing) to produce predominantly unisexual progenies but with a scattering of males. These lines are used to perpetuate the "sex-ratio" stock. It is worth noting that the males do not appear in the progeny of just a few pair matings, but occur rather uniformly in all matings (compare the columns "Pairs mated" and "Matings producing males" in Table 6). Females from line III were outcrossed to males from the original culture, and produced 96 females and no males in 4 pair matings (generation 2, Table 6). From this generation on, females of line III were crossed to males from a strain of a recessive brown-like eye color mutant. This mutant was sent to the laboratory at Columbia University by DR. A. B. DA CUNHA of the University of São Paulo, but the geographic origin of the strain in which it has arisen has been lost. This outcrossing resulted in a rapid increase of the proportion of males in the cultures (generations 3–6, Table 6), and eventual

TABLE 6

Outcrosses of different "sex-ratio" lines of Drosophila paulistorum *to males from the strains VI and XI*

Generation	Flies counted	Percent males	Pairs mated	Matings producing males
"Sex-ratio" I				
2	709	0	9	0
3	779	3.8	15	3
4	419	0	10	0
5	98	3.1	4	1
6	724	0.9	15	3
7	405	0	10	0
8	1371	0.9	15	4
9	764	0.9	8	3
10	878	1.4	16	2
11	256	0	7	0
12	502	0.7	7	3
"Sex-ratio" II				
2	74	0	3	0
3	767	3.1	9	4
4	68	0	3	0
5	812	0.1	16	1
6	119	0	6	0
7	1302	9.4	20	14
8	686	0.1	8	1
9	486	0.6	16	3
10	102	0	5	0
11	323	0	5	0
"Sex-ratio" III				
2	96	0	4	0
3	314	36.3	3	3
4	300	10.7	4	4
5	599	24.0	9	9
6	724	43.5	12	12
7	745	38.1	14	14
8	338	45.4	7	7
9	1089	40.0	14	14
10	338	45.2	7	7
11	1166	37.6	10	10
"Sex-ratio" VI				
2	1121	46.5	14	14
3	600	44.6	8	8
4	1431	45.2	18	18
5	587	44.4	12	12

disappearance of the "sex-ratio" condition. Finally, females from line VI, crossed to their brothers, produced a strain with normal proportions of the two sexes.

Table 7 gives the records of the results of outcrossing females from the strain I to males from a strain from Bucaramanga, Colombia, from Costa Rica, from Içana, Brazil, and from the same strain of the brown-like mutant which has been mentioned above. In all these crosses the "sex-ratio" condition has been lost within several generations, except that the cross to Içana males has produced a sterile F_1 progeny.

The "sex-ratio" condition in *D. paulistorum* seems, thus, much less stable than in *D. willistoni*. At any rate, most of the strains of *D. paulistorum* to which the "sex-ratio" has been outcrossed contained genetic factors which acted unfavorably with respect to the regular transmission of the causative agent of "sex-ratio."

DISCUSSION

In recent years geneticists have become acquainted with several instances of inheritance through the cytoplasm. Such inheritance occurs among animals as well as among plants, among lower as well as among higher organisms. It is instructive to compare the known properties of the "sex-ratio" in *Drosophila willistoni* and *Drosophila paulistorum* with these other instances of cytoplasmic inheritance.

Perhaps the closest analogy is found in the "killer" character described and analyzed in *Paramecium aurelia* by SONNEBORN (1951, 1954) and PREER (1948). This character depends upon the presence of self-reproducing particles ("kappa") in the cytoplasm of the infusoria. However, the "kappa" particles can multiply

TABLE 7

Outcrosses of "sex-ratio" I females to males from unrelated strains

Generation	Flies counted	Percent males	Pairs mated	Matings producing males
Bucaramanga ♂♂				
1	721	16.5	9	7
2	1339	31.5	22	21
3	661	45.5	9	9
Mutant ♂♂				
1	84	2.4	3	1
2	70	49.9	1	1
3	2028	50.7	10	10
Costa Rica ♂♂				
1	14	0	1	0
2	682	1.7	8	2
3	816	0.9	9	3
4	909	35.5	6	6
Içana ♂♂				
1	149	0	3	0

successfully only in animals which carry alleles of a certain gene, *K*, in their nuclei. Animals which lack this gene lose kappa within a certain number of cell divisions, but the presence of *K* is not itself sufficient to initiate the production of kappa in the cells which have not inherited these particles. In our "sex-ratio" strains the original progenitors evidently carried nuclear genes favorable for the reproduction of the cytoplasmic factor responsible for the death of the male zygotes, analogous to the gene *K* in Paramecium. On the other hand, the strains *abb bw*, Recife-3, and Recife-6 in *D. willistoni*, as well as the eye-color mutant strain and the Bucaramanga and Costa Rica strains in *D. paulistorum*, carry genes analogous to *k* in Paramecium, which suppress the reproduction of the cytoplasmic factors responsible for the "sex-ratio."

The admirable studies of L 'HÉRITIER and SIGOT (1946), L 'HÉRITIER and SCOEUX (1947), and L 'HÉRITIER (1951, 1955) have clarified the details of a most interesting case of cytoplasmic inheritance in *Drosophila melanogaster*, that of the sensitivity of the flies to poisoning by carbon dioxide. This character depends upon the presence in the cytoplasm of an "integrated virus" (virus intégré), which, however, can be transmitted not only through the egg cytoplasm but also, though less efficiently, through male sex cells. This last capacity is apparently not shared by the "sex-ratio" factors. On the other hand, the virus which induces the sensitivity to carbon dioxide can be transmitted from flies to flies not only through the sex cells but also, in experiments, by injection of hemolymph and by implantation of organs of the sensitive strain. It has been shown (MALOGOLOWKIN and POULSON 1957, and unpublished data), the causative agent of the "sex-ratio" can also be transmitted to previously uninfected strains by injection of the oöplasm of dying male eggs into normal females. The other property of the virus studied by L 'HÉRITIER and his collaborators, namely its low resistance to treatments with high temperatures, is not shared by our "sex-ratio" factors.

Other cases resembling the ones described in the present article are, as already stated in the introduction, those of the "sex-ratio" in *Drosophila bifasciata* (MAGNI 1952, 1954), in *D. prosaltans* (CAVALCANTI and FALCÃO 1954, and CAVALCANTI, FALCÃO and CASTRO 1957), and in *D. borealis* (CARSON 1956). However, MAGNI was able to effect a "cure" of the "sex-ratio" by high temperatures, while CAVALCANTI and FALCÃO found strains containing nuclear genes which disrupt the transmission of the "sex-ratio" particles. A further analogue is the character "extra setae on the forelegs" of the mite *Tetranychus cinnabarrinus* (Boudreaux, a report read before the Entomological Society of America in December 1956, which its author kindly permits us to mention here). MITCHELL and MITCHELL (1956) described a most suggestive case in the fungus, *Neurospora crassa*. Here a trait, "poky", is inherited through the cytoplasm, but its phenotypic manifestation is suppressed by a nuclear gene, *F*, which does not, however, destroy the cytoplasmic particle. The "sex-ratio" strains in *D. willistoni* and *D. paulistorum* show, therefore, an interesting combination of properties which are encountered separately in several other known cases of cytoplasmic inheritance.

SUMMARY

1. A female of *Drosophila willistoni* which produced a unisexual progeny consisting exclusively of daughters was found by MR. SPASSKY among 137 females of the same species collected on the island of Jamaica by Dr. W. B. HEED. Similarly, MR. SPASSKY noticed that one of the 85 females of *D. paulistorum*, collected by DR. HEED on the Sierra Nevada de Santa Marta, Colombia, produced a progeny consisting mostly of daughters and only few sons. These two females have been the progenitors of the "sex-ratio" strains of the two species, which are characterized by the production of unisexual, or nearly unisexual, female progenies.

2. The "sex-ratio" condition is inherited strictly through the female line. Females from the "sex-ratio" strains produce only or mostly daughters, regardless (with the exception noted below) of the genetic nature of the males to which they are crossed. The occasional sons which appear in the progenies of "sex-ratio" females do not differ from sons of normal females in their influence on the proportions of the sexes in their offspring.

3. The inheritance of the "sex-ratio" is due to the transmission of a causative agent through the cytoplasm of the egg. The "sex-ratio" condition is retained even when all the chromosomes of the original "sex-ratio" strain are replaced by their homologues from lines giving normal progenies. This causative agent acts by making the eggs fertilized by the Y-bearing sperms lethal.

4. The cytoplasmic factor causing the "sex-ratio" condition is, nevertheless, not independent from chromosomal genes. This is evidenced by the fact that some wild and mutant strains induce reversions to normal proportions of the sexes in the crosses to "sex-ratio" strains. However, a loss of the "sex-ratio" is irreversible; females from the progenies of flies containing about equal numbers of daughters and sons breed like normal females.

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